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## **Evaluation of Some Element and Mineral Levels in Prescription and Non-Prescription Dog Diets**

#### Key words

elements;
minerals;
dog;
food;
prescription

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**Abstract:** Various prescription diets prescribed by veterinarians for specific diseases in dogs have been developed and introduced to the market. Trace element and mineral levels, which are essential for healthy living conditions in animals, can differ in both prescription and non-prescription foods. In our study, it was aimed to determine the levels of some elements and minerals in various prescription and non-prescription dry foods used in dog nutrition and to evaluate their therapeutic importance.

In the study, a total of 100 different prescription dry food formulated for hepatic diseases (H, n=25), renal diseases (R, n=25), gastrointestinal diseases (GI, n=25) and, allergic diseases (HA, n=25) were used. Non-prescription dry foods from different flavors and brands in the market were considered as the control group (C, n=50). Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), Selenium (Se), Calcium (Ca), and Phosphorus (P) levels of all dry foods were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Thermo iCAP 6000 series) and the results were compared between groups. Statistical analysis was evaluated using SPSS 21.

Cu levels in GI and HA groups were higher than in the control group (p<0.05 and p<0.01, respectively). Fe levels were higher in the GI group and lower in the HA group than in the control group (p<0.05). Mn level was significantly higher in the H group compared to the control group (p<0.001). The Mn levels in GI and HA groups were higher than the control group (p<0.01). There was no statistical difference in Se and Zn levels between prescription and non-prescription dry foods. Ca and P levels in all groups were statistically lower than in the control group (p<0.001).

There are significant differences in element and mineral levels in prescription and nonprescription dry foods. These values may be out of the legal limits determined by EU Regulation. Considering the therapeutic purpose of these prescription formulas, some element and mineral amounts were determined as inappropriate.

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## Introduction

The growing interest in pets has led to an increase in the annual growth rate of the pet food industry. It was reported that 8.5 million tonnes of pet food products are sold

annually in Europe (1). Commercial foods are widely preferred by pet owners because they meet the nutritional needs of pets practically and economically (2). In addition to commercial foods used in healthy animals, various prescription diets formulated for many disease conditions have also been introduced to the market (3). Today, prescription foods are widely prescribed by veterinarians and many studies were on their clinical efficacy (4-10). Urinary diets effective on urinary system that contain lesser amounts of high-guality protein, low phosphorus and magnesium in order to decrease the concentration of urea, phosphorus and magnesium in the urine (4); hepatic diets include moderate fat, high carbohydrates, highly digestible, high biologic value protein that is low in aromatic amino acids and methionine and high in branched-chain amino acids and arginine (5); gastrointestinal diets with high digestibility and biological value protein; pancreatic diets with high digestibility, restricted protein and fat formulated to reduce pancreatic secretions (7); dermatological diets enriched with omega-6 EFA linoleic acid (8) hypoallergenic diets against food allergies containing lamb and rice [9] and diets developed against obesity (10) are among the prescription foods in veterinary medicine.

Trace and macro elements are essential for healthy dogs. While the deficiency of essential trace elements such as can lead to various dysfunctions and death (11,12), greater amounts of these elements such as selenium, copper and zinc may also cause various tissue and organ damage in dogs. Therefore the optimum amount of these elements in pet foods plays an important role in maintaining health conditions (13-18). In this study, it was aimed to evaluate and compare some element and mineral levels between various non-prescription dry foods used in healthy dog nutrition and prescription dry foods used in various diseases.

## **Material and Methods**

#### Study design

The study was carried out at Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Internal Medicine collaboration with Cerrahpaşa Faculty of Medicine, Department of Biophysics. Non-prescription dry food from different brands of various companies sold on the market and prescription dry food from different brands of various companies sold in veterinary clinics were collected. All food type was dry food for dog nutrition. For nonprescription foods, predominant flavours were chicken, fish and lamb manufactured by Italy and Turkey. Hepatic foods were chicken and fish flavoured manufactured by France and Italy. All renal foods were fish flavoured manufactured by Italy and Spain. Gastrointestinal foods had chicken, fish and duck protein source manufactured by Spain, Italy and France. All hypoallergenic foods were fish flavoured manufactured by Italy and Spain. In the study, a total of 100 prescription dry dog foods developed for hepatic diseases (n=25), kidney diseases (n=25), gastrointestinal system diseases (n=25) and allergic skin diseases (n=25) were used. As a control group (n=50) non-prescription dry foods from

different flavors and brands were analyzed. Accordingly, five different study groups were determined as follows:

- Control (C, n=50): Non-prescription dry dog food samples from different flavors and brands used in healthy dogs,
- Hepatic (H, n=25): Dry dog food samples used in liver diseases,
- Renal (R, n=25): Dry dog food samples used in kidney diseases,
- Gastrointestinal (GI, n=25): Dry dog food samples used in gastrointestinal system diseases,
- Hypoallergenic (HA, n=25): Dry dog food samples used in allergic skin diseases.

#### Element and mineral analysis

Element and mineral analyzes were performed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Thermo iCAP 6000 series). Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), Selenium (Se), Calcium (Ca), Phosphorus (P) were analyzed from each dry food sample. In order to analyze the elements in ICP-OES, suitable wavelengths for each element were selected (Table 1).

 Table 1: Wavelengths of each element in ICP-OES measurements

Element	Wavelengths (nm)
Cu	324.754
Fe	259.940
Mn	257.610
Zn	206.200
Se	196.090
Са	317.933
Р	177.495

In order to determine the Cu, Fe, Mn, Zn, Se, Ca, and P levels from the collected dry foods, 4 samples were prepared from each type of food and the average values of the elemental analysis results were calculated. The element levels obtained as a result of the measurements were expressed as mg/gr <sub>(sample)</sub> for Fe, Ca and P, and  $\mu$ g /gr <sub>(sample)</sub> for Cu, Mn, Zn, and Se. 1 mL nitric acid was added to the food samples, which were transferred into heat resistant graduated glass tubes, and left to melt in an oven at 200°C. Then, 1 mL of perchloric acid was added to the nitric acid food sample,

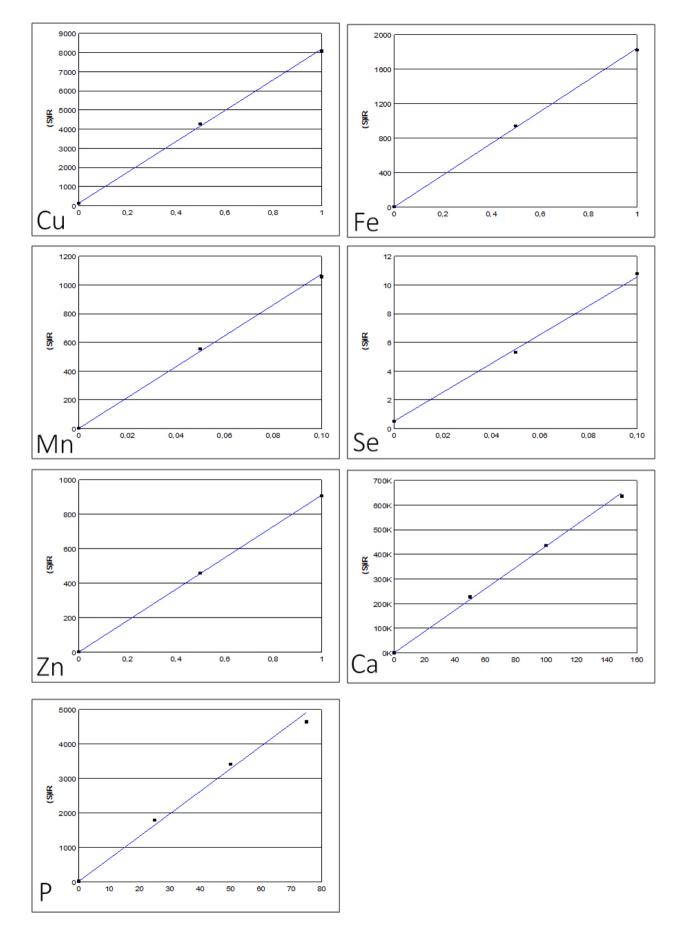


Figure 1: Calibration curves for Cu, Fe, Mn, Se, Zn, Ca and P, respectively

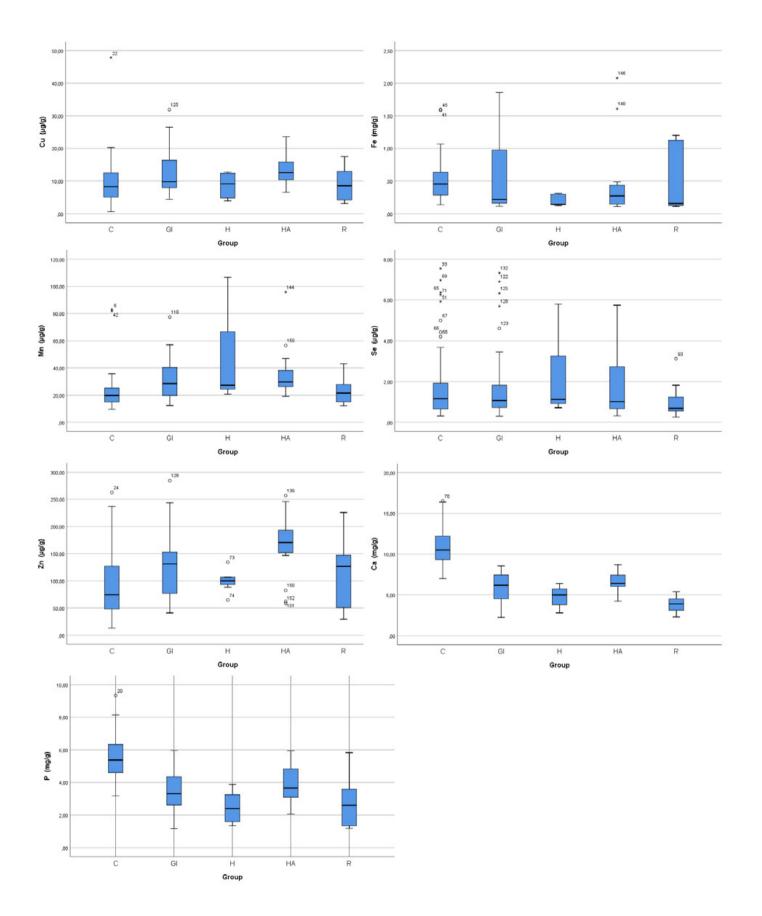


Figure 2: Box-plots for Cu, Fe, Mn, Se, Zn, Ca and P. C: Control, GI: Gastrointestinal, H: Hepatic, HA: Hypoallergenic, R: Renal

which was left to cool at room temperature and vortexed. The mixture, which was put in an oven at 200°C and a wet burning process, was vortexed and left to cool. Distilled water was added to the samples and the total volume was completed to 13 mL. It was vortexed again and analyzed in the ICP-OES device.

Working standard solutions (Chem-Lab NV) were co-prepared from standard stock solutions (1000 pg/dL) of each element. Calibration graphics for each element were drawn and evaluated using standard solutions and deionized water as a blank solution, and then measured (Figure 1). The biggest advantage of this method is that it allows to measure the main emission of many elements at the same time, as well as their emissions at 4-5 different wavelengths. Elemental concentrations in food samples prepared for measurement were determined using these standard curves. All samples were analyzed on the same day and with the same calibration in order to minimize the factors affected by temperature, humidity and device calibration.

#### Statistical analysis

SPSS 21 statistical program was for statistical analysis of the data obtained as a result of the measurements. All results were given as Mean±Standard Error. One-way Analysis of Variance (One-Way ANOVA), a parametric test, was used to compare groups with more than two homogeneous and normal distributions, and the Kruskal-Wallis test, a non-parametric test, was used to compare groups that did not have normal distribution. In interpretations, the limit of significance was accepted as p<0.05.

#### Table 2: Element values measured in different food groups

## Results

#### **Copper measurement**

Comparisons between H group (8.678±1.430), R group (9.026±1.266) and the control group (9.442±0.846) showed that there wasn't a statistically significant difference between the groups. However, there was a statistically significant increase in Cu levels in the GI (12.431±1.120) and HA groups (13.263±1.070) compared to the control group (p<0.05 and p<0.01, respectively). With the comparisons among each prescription foods, a statistically significant difference was observed between the H and HA groups and similarly between the R and HA groups in terms of Cu levels (p<0.05) (Table 2) (Figure 2). All values obtained were expressed as  $\mu g/gr_{sample}$ 

#### Iron measurement

Although the Fe levels of the control group  $(0.495 \pm 0.035)$ and R group  $(0.479 \pm 0.216)$  decreased mathematically, there was no statistically significant difference. It was determined that the Fe level in the GI group  $(0.499 \pm 0.106)$ was statistically higher than the control group, and the Fe level in the HA group  $(0.471 \pm 0.160)$  was statistically lower than the control group (p<0.05). The Fe level in the H group  $(0.202 \pm 0.042)$  was statistically lower (p<0.01) than the control group. When the prescription food groups were compared among themselves, no statistically significant difference was observed (Table 2) (Figure 2). All obtained values were expressed as mg/gr<sub>sample</sub>.

#### Manganese measurement

Statistically significant increased Mn level was observed in the H group (45.46  $\pm$  13.7) compared to control group (21.81  $\pm$  1.43) (p<0.001). In the comparison of control group

Elements Control (n=50)		H (n=25)	R (n=25)	GI (n=25)	HA (n=25)	
Cu (µg/gr <sub>(sample)</sub> )	9.442 ± 0.846	8.678 ± 1.430	9.026 ± 1.266	12.431 ± 1.120*	13.263 ± 1.070**,a,b	
Fe (mg/gr <sub>(sample)</sub> )	0.495 ± 0.035	0.202 ± 0.042**	0.479 ± 0.216	0.499 ± 0.106*	0.471 ± 0.160*	
Mn (µg/gr <sub>(sample)</sub> )	21.81 ± 1.43	45.46 ± 13.17***	22.50 ± 2.17ª	31.66 ± 2.42**,b	35.00 ± 3.74**,bb	
Zn (µg/gr <sub>(sample)</sub> )	89.44 ± 8.52	99.85 ± 6.89	112.06 ± 14.06 126.44 ± 9.87**		168.28 ± 11.89***,aa,bb,cc	
Se (µg/gr <sub>(sample)</sub> )	2.006 ± 0.297	2.286 ± 0.737	1.008 ± 0.199ª	1.894 ± 0.337	1.842 ± 0.404	
Ca (mg/gr <sub>(sample)</sub> )	10.734 ± 0.262	4.779 ± 0.451***	3.840 ± 0.205***	5.941 ± 0.282***,bbb	6.581 ± 0.271***.a,bbb	
P (mg/gr <sub>(sample)</sub> )	5.534 ± 0.152	2.465 ± 0.334***	2.634 ± 0.337***	3.446 ± 0.212***	3.897 ± 0.249***,a,b	

\*, a,b: p<0.05; \*\*, aa, bb: p<0.01; \*\*\*, aaa, bbb, ccc: p<0.001. M±S.E.:Mean ± Standard Error

H: Hepatic Diet, R: Renal Diet, GI: Gastrointestinal Diet, HA: Hypoallergenic Diet.

\*: Between control group and prescription food groups.

a: Between H group and R, GI, HA groups. b: Between R group and GI, HA groups. c: Between GI group and HA group.

compare to GI (31.66 ± 2.42) and HA group (35.0 ± 3.74), Mn levels were similarly higher in the GI and HA groups than the control group (p<0.01). There was no statistical difference in Mn levels between control and R groups. When the H, R, GI and HA groups were compared among themselves according to Mn levels, a statistically significant difference was observed in the H and R groups (45.46 ± 13.17 and 22.50 ± 2.17, respectively) (p<0.05). In addition, statistically significant differences were observed when R group compared with GI and HA groups (p<0.05 and p<0.01, respectively) (Table 2) (Figure 2). All values obtained were expressed as  $\mu g/gr_{sample}$ .

#### Zinc measurement

Although there was a mathematical difference in H (99.85  $\pm$  6.89) and R group (112.06  $\pm$  14.06) compare to control group (89.44  $\pm$  8.5), there was no statistically significant difference. However, Zn levels in the food samples of the HA (168.28  $\pm$  11.89) and Gl (126.44  $\pm$  9.87) groups were statistically higher compared to the control group (p<0.001 and p<0.01, respectively). When the H, R, GI and HA groups were compared among themselves, there was a statistically significant difference between HA group and the H, R and GI groups (p<0.01) (Table 2) (Figure 2). All values obtained were expressed as  $\mu$ g/gr<sub>sample</sub>.

#### Selenium measurement

There was no statistically significant difference in the H group (2.286  $\pm$  0.737), R group (1.008  $\pm$  0.199), GI group (1.894  $\pm$  0.337), and HA group (1.842  $\pm$  0.404) compared to the control group (2.006  $\pm$  0.297). However, a statistically significant decrease was observed in the R group compared to the H group (p<0.05) (Table 2) (Figure 2). All values obtained are expressed were  $\mu$ g/gr<sub>sample</sub>.

#### **Calcium measurement**

The Ca levels measured in the prescription dry food groups were 4.779  $\pm$  0.451, 3.840  $\pm$  0.205, 5.941  $\pm$  0.282 and 6.581  $\pm$  0.271 for the H, R, GI and HA, respectively. Ca levels in all groups were statistically lower than the control group (10,734  $\pm$  0.262) (p<0.001). There were statistically significant differences between the H group and the HA (p<0.05) group; between the R group and the GI and HA groups (p<0.001) (Table 2) (Figure 2). All obtained values were expressed as mg/gr<sub>sample</sub>.

#### Phosphorus measurement

The P levels measured in the prescription dry food groups were  $2.465 \pm 0.334$ ,  $2.634 \pm 0.337$ ,  $3.446 \pm 0.212$  and  $3.897 \pm 0.249$  for the H, R, GI and HA groups, respectively. The P level in all groups was lower than the control group (5.534  $\pm 0.152$ ) (p<0.001). There was a significant difference between the H group and HA (p<0.05) and between the R group and the HA group (p<0.05) (Table 2) (Figure 2). All obtained values were expressed as  $mg/gr_{sample}$ .

## Discussion

#### **Copper Levels**

Cu is an essential micronutrient for all living organisms. All pet foods originated from vegetable or animal based protein contain Cu. Cu uptake occurs mainly through the digestive system. Although Cu absorption is regulated by enterocytes, it is mainly taken into the organism through food. Experimental studies have shown that chronically high dietary Cu intake leads to Cu accumulations in the liver (19). It is known that hepatic Cu accumulations cause hepatocellular necrosis, chronic hepatitis, cirrhosis and inflammation in cats and genetically susceptible dog breeds (Bedlington terrier, West Highland White Terrier, Skye Terrier, Dalmatian) (20-22).

In the current recommendation of FEDIAF published in 2021, the minimum value for Cu in adult dog foods is 0.83 - 0.72 mg (95 kcal - 119 kcal per kg) per 100 gr dry matter, minimum 1.10 mg and maximum 2.80 mg (legal limit) in early and late growth periods in puppies. Cu values measured in both prescription and non-prescription foods in our study are consistent with the recommended amounts (Table 3). Cu levels in prescription foods used in various kidney and liver diseases and non-prescription foods used in healthy dogs were measured at similar amounts. Therefore, a food-related Cu deficiency unlikely occurs in dogs fed long-term with these foods. 48% (24/50) for non-prescription, 16% (4/25) for HA, 32% (8/25) for GI, 52% (13/25) for H and 56% (14/25) for R group revealed non-compliance with FEDIAF Guidelines in terms of minimum level. Only for non-prescription group, 2% (1/50) were greater than the maximum legal limit.

Cu acts as a cofactor for many antioxidant enzymes (23). In the case of gastroenteritis of various etiologies, decreases in plasma Cu levels may be observed due to Fe and Cu lost with diarrhea (23, 24). In our study, the Cu level in Gl group was higher than non-prescription dog foods. This result confirms that the use of Gl foods, especially in dogs affected by severe and hemorrhagic diarrhea, is more appropriate compare to non-prescription dog food in terms of compensating the potantial Cu deficiency.

Dry foods included in the HA group are a diet formulated with selected protein and carbohydrate sources to reduce sensitivity to nutrients in dogs. In our study, the Cu level in the HA group was significantly higher than the non-prescription formulas. Since the quality of the protein used for allergy elimination is substantial in hypoallergenic formulas, the high amount of Cu content may be insignificant considering its purpose of use. In fact, many studies in humans have reported that Cu may cause hypersensitivity in people (25-27). Therefore, the higher Cu level in HA group compared to the non-prescription formulas may not be appropriate and may contribute to allergic reactions.

#### Iron Levels

Some of the Fe additive sources in commercial dog foods are in the form of Dicalcium phosphate (1.4% Fe) and Ferrous sulfate heptahydrate (21.8% Fe) (28). According to FEDIAF Nutritional Guidelines 2021, the minimum Fe level is determined as 3.60 mg (for 110 kcal/kg), 4.17 mg (for 95 kcal/kg) in 100 gr dry matter in adult dog foods. Legal upper limit is 68.18 mg/100 gr dry matter. Comparing the FEDIAF recommended limits and the results of our study, Mean Fe levels in all food groups was measured within the legal limits (Table 3). However 16% (8/50) for non-prescription, 8% (2/25) for HA, 20% (5/25) for GI, and 12% (3/25) for R group revealed non-compliance with FEDIAF Guidelines in terms of maximum level.

National Research Council (NRC) declared in 2006 that there is no suitable data for determining the safe upper limit for Fe. In addition, it is taken into account that some Fe sources cannot be able to used in some animals. For this reason, For this reason, it is stated that the use of Fe in food production is aimed for coloring rather than nutrition. As a result, although the exact amount of Fe required for dogs is not known, it is emphasized that producers should be aware that excessive iron intake out of the recommended values may be toxic (AAFCO 2014) (29).

Although there were no significant differences in mean Fe levels between formulas, the mean Fe value in prescription formulas used in allergic and liver diseases (HA and H) was lower than non-prescription formulas, and higher in formulas used in gastrointestinal diseases (GI). Fe, like Cu, is essential for normal cellular functions. Excessive intake of Cu and Fe leads to oxidative damage, resulting in hepatocyte loss and inflammation in the liver (30). Therefore, lower Fe levels may be expected in hepatic prescription diets compared to non-prescription diets. However, the relatively high level of Fe in GI foods, which may be preferred especially in cases of diarrhea or hemorrhagic diarrhea, may be important in compensating the possible Fe loss with hemorrhagic diarrhea.

In some studies in human medicine, the association of low Fe concentrations with allergic conditions such as atopic dermatitis and eczema in humans has been reported (31-33). Although this relationship is not clear enough in cats and dogs, according to the results of our study, it may be significant that the Fe level in HA group foods is lower than in non-prescription foods.

#### Manganese Levels

According to FEDIAF, Mn values are minimum 0.58 mg (110 kcal/kg) and 0.67 mg (95 kcal/kg); legal upper limit is 17 mg in 100 gr dry matter for adult dogs. In our study, the mean Mn values in all formulas were between the legal limits determined by FEDIAF (Table 3). None of the groups were revealed non-compliance with FEDIAF Guidelines in terms of minimum and maximum level.

Mn levels were significantly higher in H, GI, R and HA groups compared to non-prescription group. Mn concentration is controlled by the liver. In human medicine, an increase in serum Mn concentration in patients with liver failures, decreased portal perfusion, cirrhosis and congenital portosystemic shunts (PSS) therefore, accumulations in the

Table 3: Comparison of mean trace element and mineral values in 100 gr dry matter for H, R, GI, HA and non-prescription food groups with minimum and maximum values determined by FEDIAF

Trace elements	Minimum Recommended Level for adults (95 kcal - 110 kcal per kg)	Maximum Recommended – Level (Legal limit)	Mean measured element levels in H, R, GI, HA and N-P groups respectively				
			Н	R	GI	НА	Control
Cu (mg)	0.83 - 0.72	2.80	0.86	0.9	1.24	1.32	0.94
Fe (mg)	4.17 - 3.60	68.18	20.2	47.9	49.9	47.1	49.5
Mn (mg)	0.67 - 0.58	17.00	4.5	2.2	3.1	3.5	2.1
Zn (mg )	8.34 - 7.20	22.70	9.9	11.2	12.6	16.8	8.9
Se (µg)	22.00 - 18.00	56.80	228.6	100.8	189.4	184.2	200.6
Ca (gr)	0.58 - 0.50	2.50	0.47	0.38	0.59	0.65	1
P (gr)	0.46 - 0.40	1.60	0.24	0.26	0.34	0.38	0.55

H: Hepatic Diet, R: Renal Diet, GI: Gastrointestinal Diet, HA: Hypoallergenic Diet

associated tissues and organs was reported (34). It has been suggested that due to the inability to remove Mn from the liver, Mn level may be also high in dogs with congenital PSS (35). In our study, it was observed that the Mn level was the highest in the formulas used in the treatment of liver diseases compared to other prescription diet foods, and this elevation was significant at the p<0.001 level compared to the control group. As a result, we think that high Mn levels in hepatic formulas may pose a significant risk due to possible Mn accumulations in liver diseases.

About 5% of Mn is distributed from the plasma to the kidney. Therefore, exposure to excessive Mn levels can cause kidney dysfunction. Both in vivo and in vitro studies have shown that high levels of Mn exposure are associated with renal dysfunction (36). The Mn level measured in this study was inappropriately high in renal formulas compared to non-prescription formulas. Manganese superoxide dismutase has been defined in the IgE-reactive autoantigens and its allergic role has been reported in some studies (37,38). Therefore, lower Mn levels may be expected in the HA group compared to the other groups in the study.

#### Zinc Levels

According to a study conducted in 1991, the minimum value for Zn determined by the National Research Council was 39  $\mu$ g/gr (39), whereas today the minimum Zn value in adult dog foods is 7.2 mg (110 kcal/kg) per 100 gr dry matter and 8.34 mg (for 95 kcal/kg); legal upper limit is 22.7 mg per 100 gr dry matter according to FEDIAF. In our study, Mean Zn was measured within the determined limits in all study groups (Table 3). 28% (14/50) for non-prescription, 12% (3/25) for HA, 24% (6/25) for GI, 12% (3/25) for H and 32% (8/25) for R group revealed non-compliance with FEDIAF Guidelines in terms of minimum level. 2% (1/50) for non-prescription, 16% (4/25) for HA, 4% (1/25) for GI, 8% (2/25) for R group revealed non-compliance with FEDIAF Guidelines in terms of maximum level.

The highest Zn level (168.28  $\mu$ g/gr) was obtained in foods used in allergic skin diseases. It was found that the Zn values in the formulas used in gastrointestinal diseases and allergic diseases were significantly higher, at p<0.01 and p<0.001, respectively, compared to the control group. Zn deficiency may cause gastrointestinal diseases characterized by diarrhea and loss of appetite (40). Considering the important effects of Zn on the improvement of skin diseases and hair growth, the higher level of Zn in diets used in allergic skin diseases compared to the control group and other prescription formulas may contribute to the treatment of allergic skin diseases.

It is also reported that supplemental zinc in dogs stimulates hair growing (41). In a study conducted by Or et al. on 71 dogs (42), it was reported a correlation between low Zn levels and skin diseases. In addition, Zn provides membrane stabilization by preventing Cu accumulation and fibrosis formation in the liver. It also has free radical scavenging and antioxidant effects. Therefore, Zn supplements are recommended in liver diseases and conditions associated with hepatic Cu accumulation (35). In a study on Labrador Retriever dogs, it was reported that there was no data on the potential effect of dietary Cu and Zn on hepatic Cu and Zn levels (21). However, in our study, the lowest mean Zn level among the prescribed diet foods was obtained from the formulas used in liver diseases.

#### Selenium Levels

According to FEDIAF data, the legal limits of Se value in 100 gr of dry food in adult dog foods are minimum 18 µg for 110 kcal/kg and 22 µg for 95 kcal; maximum 56.80 µg/g per 100 gr dry matter. In our study, Se levels in all formulas were above the legal limits determined by FEDIAF (Table 3). 76% (38/50) for non-prescription, 84% (21/25) for HA, 80% (20/25) for GI, 100% (25/25) for H and 68% (17/25) for R group revealed non-compliance with FEDIAF Guidelines in terms of maximum level.

No data are available to accurately indicate the amount of Se requirement in adult dogs. According to the European Union legislation, the maximum legal limit for Se as a food additive is  $0.5 \mu g/gr$  (43). Se bioavailability is affected by the Se form (selenite, selenate, selenocysteine, selenomethionine etc.), animal species, content of the food. Selenomethionine is known as the most bioavailable Se form. The optimal Se concentration may vary due to different Se forms and bioavailability. It has beneficial effects on healthy skin and joint, hair structure, immune resistance and antioxidant properties. Se deficiency in dogs leads to disorders associated with myopathies. It has been reported that Se deficiency causes myocardial necrosis in young people and myodegenerations in adults, and plays an important role in hair growth (44).

Although not statistically significant compared to the control group, the highest mean Se value among the groups was obtained from the foods used against liver diseases (2.286  $\mu q$  /q). In our study, the average Se value obtained from foods for allergic skin diseases was measured as 1.842 µg /g. Considering its positive effects on skin and hair growth, high amount of Se in foods used in allergic skin diseases may be considered appropriate. However, in our results, although it was not statistically significant compared to others, a low Se level was obtained in diet used in dermatology. The mean amount of Se detected in foods recommended kidney diseases was lower than in hepatic foods (p<0.05). However, several human studies have shown a correlation between renal failure and low Se concentrations. Therefore, adequate dietary Se intake can be expected to have a positive effect on kidney damage (45-47).

#### **Calcium Levels**

Ca is an essential mineral plays both structural and functional roles in cats and dogs. These include bone and tooth formation, coagulation mechanism, and neural transmission (48). The Ca value in adult dogs is stated as minimum 5 gr for 110 kcal/kg and 5.8 gr for 95 kcal/kg; maximum upper limit is 2.5 gr in 100 gr dry matter according to FEDIAF. In our study, Ca levels in renal and hepatic diets were measured below the determined minimum values. In non-prescription foods mean Ca level was between the legal limits (Table 3). 16% (4/25) for HA, 32% (8/25) for GI, 48% (12/25) for H and 92% (23/25) for R group revealed non-compliance with FEDIAF Guidelines in terms of minimum level. Ca level was significantly lower in all prescription food groups compared to the control group. In dogs and cats with chronic renal failure, an increase in ionized Ca levels and consequently hypercalcemia usually occurs with disturbances in Ca homeostasis (49). Accordingly, Ca restriction is expected in renal diets.

#### **Phosporus Levels**

The P value in adult dogs is stated as minimum of 0.4 gr for 110 kcal/kg and 0.46 gr for 95 kcal/kg; maximum 1.6 gr as a legal limit according to FEDIAF. In our study, P levels in non-prescription market foods used in healthy dogs were between the lower and upper limits determined by FEDIAF. However in all prescription foods mean P levels were lower than minimum recommended level determined by FEDIAF (Table 3). 10% (5/50) for non-presciption, 56% (14/25) for HA, 64% (16/25) for GI, 100% (25/25) for H and 92% (23/25) for R group revealed non-compliance with FEDIAF Guidelines in terms of minimum level.

In small animals P is one of the most important indicators in chronic renal failure. Restriction of dietary P intake slows the progression of kidney damage. Therefore, it is recommended to significantly limit the P level in dry foods used in renal diseases. P values in all prescription formula groups are statistically lower than the control group. It is appropriate that the renal prescription formula contains lower P than the other prescription formulas. However, it should be taken into account that the use of long-term prescription diets may cause phosphorus deficiency and accordingly secondary diseases in dogs.

In a study comparing some element values of various pet foods in UK and FEDIAF guideline, it was reported a broad inconsistency in dog foods (61%) (50). In our study, the mean values showed consistent results with FEDIAF report except for Se, Ca and P. However similar with the previous study (50), when each dietary foods were evaluated individually, high number of incompatibility with FEDIAF was observed. Se was measured greater than the upper limits of FEDIAF in all food groups. P was lower than both determined minimum limits and non-prescription dry foods. In a study investigating some trace element values in pet foods, Cu, Fe and Mn levels (min-max) were measured as 3.33-16.6, 23.9-71.1, and 3.28-24.4, respectively ( $\mu$ g/gr) (51). Cu values are consistent with our results. Mean Cu levels were measured minimum 8.67  $\mu$ g/gr and maximum 13.26  $\mu$ g/gr in all our food groups. Similarly, in this study, mean Mn levels (minimum 21.8  $\mu$ g/gr and maximum 45.4  $\mu$ g/gr) show compatibility with the study of Duran et al. However Fe was measured higher in all our study groups.

## Conclusions

Some element and mineral values show significant differences between prescription and non-prescription market foods. Concentrations of these elements in formulas should be reconsidered, since Mn is measured higher in hepatic and renal formulas compared to the control group, and Zn is lower in hepatic formulas compared to the control group. We think that high Mn levels in hepatic renal formulas may pose a significant risk due to possible Mn accumulations in liver and kidney diseases. Se was measured greater than the upper limits of FEDIAF in all food groups. P was lower than both determined minimum limits and non-prescription dry foods. Our results were similar with the previous studies related to high Se values in pet foods. In our opinion, the upper and lower limits of trace element and mineral contents of pet foods should be reconsidered.

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Ethical statement. This is an observational study. Trakya University, Local Ethics Committee of Animal Experiments has confirmed that no ethical approval is required (28.11.2016/TUHADYEK-2016/47).

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# Vrednotenje vsebnosti nekaterih elementov in mineralov v predpisani in nepredpisani prehrani za pse

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**Izvleček:** Na trgu so prisotne različne diete na recept, ki jih veterinarji predpisujejo za določene bolezni psov. Vsebnost elementov v sledovih in mineralov, ki so bistveni za zdravo življenje živali, se lahko razlikuje tako v hrani na recept kot v hrani brez recepta. V naši študiji smo želeli določiti vsebnost nekaterih elementov in mineralov v različnih predpisanih in nepredpisanih suhih vrstah hrane za pse, ter oceniti njihov terapevtski pomen.

V študiji je bilo uporabljenih 100 različnih vrst suhe hrane, formulirane za jetrne bolezni (H, n=25), ledvične bolezni (R, n=25), bolezni prebavil (GI, n=25) in alergijske bolezni (HA, n=25). Suha hrana brez recepta različnih okusov in blagovnih znamk na trgu je bila obravnavana kot kontrolna skupina (C, n=50). Vsebnost bakra (Cu), železa (Fe), mangana (Mn), cinka (Zn), selena (Se), kalcija (Ca) in fosforja (P) v vseh vrstah suhe hrane smo analizirali z optično emisijsko spektroskopijo z induktivno sklopljeno plazmo (ICP-OES, serija Thermo iCAP 6000) in rezultate primerjali med skupinami. Statistična analiza je bila narejena v programu SPSS 21.

Vsebnost Cu v skupinah GI in HA je bila višja kot v kontrolni skupini (p<0,05 oziroma p<0,01). Vsebnost Fe je bila višja v skupini GI in nižja v skupini HA kot v kontrolni skupini (p<0,05). Raven Mn je bila bistveno višja v skupini H v primerjavi s kontrolno skupino (p<0,001). Ravni Mn v skupinah GI in HA so bile višje kot v kontrolni skupini (p<0,01). Med suho hrano na recept in suho hrano brez recepta ni bilo statistične razlike v vsebnosti Se in Zn. Vsebnosti Ca in P so bile v vseh skupinah statistično značilno nižje kot v kontrolni skupini (p<0,001).

Vsebnost elementov in mineralov v suhi hrani na recept in suhi hrani brez recepta se je bistveno razlikovala. Te vrednosti so lahko izven zakonsko določenih mejnih vrednosti, ki jih določa uredba EU. Glede na terapevtski namen diete na recept smo nekatere količine elementov in mineralov določili kot neustrezne.

Ključne besede: elementi; minerali; pes; hrana; recept