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LABRADOR KÖPEK YAVRULARININ MAMALARINA İLAVE EDİLEN ÇİNKONUN ETKİLERİ
EFFECTS OF SUPPLEMENTAL ZINC TO THE DIET OF LABRADOR PUPPIES

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ABSTRACT
The objective of the present work was to evaluate the effects of supplemental zinc (Zn) to the diet of puppies on live weight changes, skeletal growth parameters, and some of serum metabolites. Forty, eight-week-old Labrador Retriever puppies were divided into two groups (n=20) and were fed a mix of commercial dry dog food, cow milk and boiled egg with (treatment) or without (control) 100 ppm dietary supplemental zinc sulphate ($ZnSO_4$) for 8 weeks. Supplementing 100 ppm Zn to the diet of puppies did not influence body weights (p=0.403). Although insignificantly, puppies consuming a diet supplemented with Zn had greater body heights, body lengths, hearth girths, widths of chest, and widths of hip (p=0.097). Puppies on a diet supplemented with Zn had lower calcium (Ca) (p=0.075), magnesium (Mg) (p=0.013), copper (Cu) (p=0.061), but greater cholesterol (p=0.038), glucose (p=0.010), and Zn (p=0.017) serum concentrations compared with puppies fed a diet not supplemented with Zn. The results of the present study showed that supplementing 100 ppm zinc to the diet of Labrador puppies did not change weight gains but, although not greatly, promoted skeletal growth. Serum parameters of clinical importance were influenced by Zn supplementation.

Key words: Zinc, puppies, Labrador, growth

ÖZET
Bu çalışmanın amacı köpek yavruları mamalarına katılan çinkonun (Zn) canlı ağırlık değişimi, iskelet büyüme parametreleri ve kimi serum parametrelerine etkisini araştırmaktı. 40 adet sekiz haftalık yaşta Labrador Retriever cinsi köpek yavruları iki eşit sayıda (n=20) gruba ayrılarak sekiz hafta süreyle ticari bir köpek maması, inek sütü ve haşlanmış yumurta ile birlikte 0 (kontrol) veya 100 ppm çinko sülfat ($ZnSO_4$) ilaveli (Uygulama) diyet tüketmeleri sağlanmıştır. Köpek yavruları diyetine ilave edilen 100 ppm Zn vücut ağırlığını etkilememiştir (p=0.403). Önemli olmamasına rağmen, Zn takviyeli diyet tüketen köpek yavruları daha büyük vücut yüksekliği, vücut uzunluğu, göğüs çevresi, kalça genişliği ve omuz genişliğine sahip olmuşlardır (p=0.097). Mamalarına Zn katılan köpek yavrularına ait serumlarda düşük kalsiyum (Ca) (p=0.075), magnezyum (Mg) (p=0.013), bakır (Cu) (p=0.061), ancak daha yüksek kolesterol (p=0.038), glikoz (p=0.010) ve Zn (p=0.017) konsantrasyonları tespit edilmiştir. Çalışma sonuçlarına göre köpek yavruları mamalarına ilave edilen 100 ppm Zn canlı ağırlığı değiştirmez iken önemsizde olsa iskelet gelişimini desteklemiştir. Klinik öneme ait serum parametreleri mamalarına Zn katılan köpek yavrularında değişime uğramıştır.

Anahtar kelimeler: Çinko, köpek yavrusu, Labrador, büyüme

INTRODUCTION
Zinc (Zn) is an essential trace element for humans and animals. More than 70 zinc metalloenzymes are required for carbohydrate, protein, lipid, and nucleic acid metabolism (1). Zinc is involved in controlling of blood glucose concentration through functioning in glucagon secretion, and insulin packaging, secretion and signaling (2,3). In addition, Byun et al. (4) stated that a zinc-binding protein (metallothionein-3) in the brain of male mice may be involved in central leptin signaling and the consequent increase in peripheral energy expenditure, playing a crucial role in the development of obesity. Other zinc metalloproteins are involved in diverse processes such as cell signaling, gene expression, membrane structure and function, and modulation of the redox state of the cell and cellular respiration (5). Zinc is also involved in immunity and chronic diseases, such as cancer, diabetes, depression, infertility, and age-related diseases (6). Indicated unusual Zn requirements of such human tissues as pancreas, prostate, and mammary gland which accumulate abundant Zn into secretory vesicles and tightly regulate Zn secretion to provide Zn

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for critical biological processes (7).

Feeding zinc-inadequate diet to animals causes a reduction in both food intake and growth (8). Zinc has an important impact on somatic growth. The pituitary, as the source of growth hormone, contains a higher concentration of zinc than other organs, and zinc enhances pituitary hormone function (9). Circulating growth hormone concentrations in rats have been reported to decrease with Zn deficiency (10). Serum insulin-like growth factor-I (IGF-I) has also been found to be low in rats fed a zinc-deficient diet (11).

Studies on Zn supplementation to puppies in terms of growth are limited in the literature. Booles et al. (12) and Wedekind and Lowry (13) reported no differences in body weights of the puppies fed a diet supplemented with different levels of Zn. The current NRC (14) recommendations of dietary zinc allowances for growing puppies are 100 ppm. However, determining the optimal Zn requirements for growing puppies is still a challenge because Zn interacts with several macro and micro nutrients, and has various responses with different bioavailability and dose of Zn sources (12,13,15). Therefore, the objective of the present work was to evaluate the effects of supplemental zinc sulfate at 100 ppm to the diet of Labrador puppies for eight weeks on live weight changes, skeletal growth parameters, and some of serum metabolites.

MATERIALS AND METHODS

A total of 40 Labrador Retriever puppies (32 males and eight females) at the age of eight weeks (after weaning) were included in the study. The puppies were both black and yellow. The dogs were all healthy and received a routine vaccine and anti-parasitic drugs before the beginning of the work. All dogs were registered with their pedigree in Turkish Military Forces (Nevsehir) and were grown for search of narcotics, rescue team, and other purposes.

The dogs were divided into two equal groups based on their initial body weights, which were similar between the groups (7.07 ± 0.32 kg vs 6.59 ± 0.44, p= 0.41). The first group served a control and the second group as treatment. The two groups both contained 16 male and four females. Each main group of animals had four subgroups containing four males and one female each.

Animals were randomly assigned to one of two dietary treatments. The dogs in control group were fed a mixture of a dry commercial diet (Petfood, Istanbul) for puppies, hard-boiled egg, and cow milk. The dogs in the treatment group received the same diet but an addition of zinc supplement of 100 ppm to their diet. Zinc was fist top-dressed then mixed well with the rest of the diet ingredients. Zinc source used as a supplement was zinc sulfate ($ZnSO_4.7H_2O$). In the first four weeks of the trial, each dog in both groups consumed 250 grams of commercial dry dog food (PetFood, Istanbul) plus a whole boiled egg (without shell, 60 gram) and 250 ml of cow milk (3.5% fat). In the second four weeks of the trial, each dog in both groups consumed 400 grams of same commercial dry dog food plus a boiled egg (without shell, 60 gram) and 400 ml of cow milk (3.5% fat). The commercial diet, egg and cow milk were mixed together before feeding. Group feeding was practiced in each subgroup. Diets were fed with two equal amounts at 07:00 and 17:00. The diets were completely consumed by the dogs of both groups. The dogs of two groups were allowed free access to tap water. The ingredients and chemical composition of the commercial diet is given in Table 1. The diets were prepared to meet or slightly exceed the nutrient requirements recommended by the National Research Council (14).

Daily Zn intakes of the puppies were 56.6 and 156.6 mg for the first four weeks, and 90.2 and 190.2 mg for the second four weeks for control and treatment groups, respectively. Calculations were made based on the declaration of commercial dry dog food label and USDA National Nutrient Database for Standard Reference Release 24. Based on these references commercial dry dog food, boiled egg, and whole milk contain 220, 10, and 4 mg/kg Zn, respectively.

With an initial adaptation period of seven days to the relative diets, the trial was conducted. During the adaptation period of the zinc-supplemented diet resulted in a reduction of feed intake and a slight diarrhea for a few days. However, after the adaptation period, feed intake and the status of diarrhea remained normal and similar to those of dogs in control group.

The animal experiment was approved by the Erciyes University Animal Ethics Committees (Date: 11 August 2010–Number: 10/57) for the use and care of experimental animals in research. The animals were reared at the temperature of 22±2^{o}C and light was provided, additional to the daylight, until 22 h every day. The dogs were kept in dog shelters with dimensions of 2x2 x2m per five dogs. The study took eight weeks and was conducted from February to April of 2011.

At the beginning of the study and every week during eight weeks, the body weight changes and skeletal growth parameters were recorded. Skeletal growth parameters included body height (point of shoulder to the floor), body length (the nape of the neck to the base of the tail), heart girth, width of chest (the straight line at shoulders), and width of hip (the straight line between femoral greater trochanter) described by Tepeli et al. (16) and Palmer (17).

At the end of the study, 14 hours from the last meal, blood samples from vena cephalica of each puppy were taken, and sera was prepared and stored at −20°C for later determination. Serum samples were thawed at room temperature and were analyzed for calcium (Ca), phosphorous (P), magnesium (Mg), iron (Fe), copper (Cu), zinc, glucose, total protein, triglyceride, cholesterol, and albumin concentrations, also lipase, creatine kinase (CK), alanine transaminase (ALT), and aspartate aminotransferase (AST) enzyme activities using commercial kits (Biolabo-France, Teco-USA, Quimica-Spain, Far-Italy) in a spectrophotometer (Shimadzu UV -1700).

The data were analyzed using Student's t-test (one-tailed), and $p<0.05$ was considered significant. The statistical analyses were performed using the SAS package (18).

RESULTS

Table 1. Composition of commercial dry dog food fed to Labrador puppies*

Component	Amount
Energy, kcal/kg ME	3850
Crude protein, %	≥ 26
Crude fat, %	≥ 17
Crude fiber, %	≤ 3.5
Crude ash, %	≤ 7.5
Water, %	≤ 10
Sodium chloride, %	≤ 1.0
Vitamin A, IU/kg	≥ 12000
Vitamin D_3, IU/ kg	≥ 1200
Vitamin E, mg/kg	≥ 300
Vitamin K_3, mg/g	≥ 2
Vitamin B_1, mg/kg	≥ 9
Vitamin B_2, mg/kg	≥ 10
Vitamin B_6, mg/kg	≥ 7
Vitamin B_{12}, mg/kg	≥ 40
Vitamin C, mg/kg	≥ 200
Niacin, mg/kg	≥ 40
Biotin, mg/kg	≥ 0.8
Folic acid, mg/kg	≥ 1
Pantothenic acid, mg/kg	≥ 30
Choline, mg/kg	≥ 2000
Magnesium, mg/kg	≥ 1000
Iron, mg/kg	≥ 170
Cobalt, mg/kg	≥ 35
Zinc, mg/kg	≥ 220
Selenium, mg/kg	≥ 0.5
Iodine, mg/kg	≥ 4
Copper, mg/kg	≥ 10

*Declaration of the chemical composition by the commercial company, Petfood, Istanbul.

Body weight changes and skeletal growth parameters of the puppies are given in Table 2. Including 100 ppm supplemental Zn in the diet of puppies did not influence body weights (p=0.403). Puppies consumed diet with or without Zn supplementation had similar body heights (p=0.374). Initial and final body length of puppies also remained similar between puppies consumed diet with or without Zn supplementations (p=0.369). However, when considered the differences between initial and final body lengths, there was a tendency (P=0.097) that puppies consuming diet supplemented with 100 ppm Zn compared with those of puppies not supplemented with Zn had greater body lengths. Supplementing Zn to the diet of puppies did not change either hearth girth (p=0.694) or width of chest (p=0.204). Initial and final hip width of puppies did not change between puppies consumed diet with or without Zn supplementations (p=0.147). However, when considered the differences between initial and final hip width, puppies consuming diet supplemented with 100 ppm Zn compared with those of puppies not supplemented with Zn tended to have greater hip width (p=0.109).

Table 3 indicates the effects of supplemental Zn on some of serum metabolites, trace elements and enzyme activities. Puppies fed a diet supplemented with Zn compared with those of puppies fed control diet had lower serum Ca (p=0.075) but similar P (p=0.808) concentrations. Supplemental Zn in the diet resulted in decreased serum Mg concentrations (p=0.013). Supplementing Zn in the puppies diet caused numerically but not statistically greater (p=0.391) serum triglyceride concentrations but significantly greater (p=0.038) serum cholesterol concentrations. All puppies in both groups had similar serum concentrations of total protein and albumin (p=0.435). Serum glucose concentrations were greater (p=0.010) in puppies fed a diet supplemented with Zn.

Serum Cu concentrations were lower (p=0.061) in puppies fed a Zn-supplemented diet but Fe concentrations were similar (p=0.472) between the two groups of puppies. Supplementing Zn to diet of puppies resulted in a greater (p=0.017) Zn serum concentrations. Serum lipase (p=0.857), CK (p=0.383), ALT (p=0.760), and AST (p=0.758) enzyme activities were similar between the puppies fed a diet with or without Zn supplementation.

DISCUSSION

Supplementing Zn to the diet of Labrador puppies for eight weeks did not change live weights. Similar to the results of the present work, (12) fed Labrador puppies a diet containing either 50 or 200 ppm Zn for 140 days and found no differences between the body weights of the puppies. Supplemented 42 ppm Zn propionate or 70 ppm Zn oxide to the diet of zinc-deficient puppies and found no differences in live weighs at the end of third week trial (13). Being parallel to the results of the body weigh changes, skeletal growth parameter namely body height, length, heart girth, hip width, and chest width remained similar between the puppy groups. Analyzed the growth rates of the different breeds of dogs and found that Labrador Retrievers as a large breed of dog have about 28 kg as adult body weight. Labrador Re

Table 2. Effects of 100 ppm dietary zinc ($ZnSO_4$) supplementation on body weights and skeletal growth parameters in Labrador puppies*

	Treatment		
	Control (± SEM; *n* = 20)	Control+$ZnSO_4$ (± SEM; *n* = 20)	*P*
Body Weight, kg			
Initial	7.07 ± 0.32	6.59 ± 0.44	0.403
Final	16.02 ± 0.67	15.58 ± 0.87	0.695
Difference	8.96 ± 0.53	8.99 ± 0.50	0.965
Body Height, cm			
Initial	25.13 ± 1.18	23.74 ± 0.91	0.374
Final	42.30 ± 0.45	41.44 ± 0.92	0.426
Difference	17.17 ± 0.82	17.17 ± 0.57	0.605
Body Length, cm			
Initial	32.03 ± 1.28	30.55 ± 0.96	0.369
Final	44.45 ± 0.80	45.09 ± 0.71	0.564
Differences	12.42 ± 0.82	14.53 ± 0.82	0.097
Heart Girth, cm			
Initial	44.13 ± 0.58	44.13 ± 1.06	0.997
Final	61.85 ± 0.50	62.24 ± 1.22	0.784
Differences	17.72 ± 0.62	18.11 ± 0.74	0.694
Width of Chest, cm			
Initial	13.83 ± 0.17	13.74 ± 0.28	0.796
Final	20.25 ± 0.22	20.77 ± 0.30	0.204
Differences	6.42 ± 0.34	7.03 ± 0.32	0.216
Width of Hip, cm			
Initial	14.00 ± 0.24	13.47 ± 0.24	0.147
Final	19.38 ± 0.42	19.76 ± 0.38	0.524
Difference	5.38 ± 0.46	6.29 ± 0.27	0.109

*Initial and final measurements were taken at the age of 8 and 16 weeks, respectively. Each treatment group contained 16 males and 4 females.

trievers reach 50% of maximum growth at 18.6 weeks, and 99% of adult body weights at 52.1 weeks (19). The body weights of the Labrador puppies at 8 and 16 weeks of the present study are in agreement with the report of the (19).

Zinc can increase bone formation through increasing osteoblast cell growth (20). Apparently, Zn supplementation of the present study, in terms of dose and source, supported osteoblast cell growth but was not high enough to cause any differences in skeletal growth. Although not statistically significant, skeletal growth parameters were numerically greater in puppies supplemented with Zn. Supplementation of dietary zinc should not probably result in a tremendous increases in body weights and skeletal sizes in puppies because faster growth may not be desirable due to complication of fast

Table 3. Effects of 100 ppm dietary zinc ($ZnSO_4$) supplementation on some serum parameters in Labrador puppies*

	Treatment		
	Control	Control+$ZnSO_4$	*P*
	(± SEM; n = 20)	(± SEM; n = 20)	
Ca, mg/dl	12.97 ± 0.69	11.14 ± 0.63	0.075
P, mg/dl	8.07 ± 0.16	8.21 ± 0.42	0.808
Mg, mEq/dl	1.76 ± 0.12	1.15 ± 0.16	0.013
Triglyceride mg/dl	53.21 ± 6.70	62.39 ± 7.58	0.391
Cholesterol, mg/dl	190.83 ± 2.90	203.71 ± 4.38	0.038
Total protein, g/dl	6.14 ± 0.07	6.29 ± 0.15	0.435
Glucose, mg/dl	105.33 ± 4.11	120.63 ± 2.93	0.010
Albumin, g/dl	4.93 ± 0.13	4.98 ± 0.11	0.752
Cu, µg/dl	87.88 ± 25.78	35.04 ± 8.34	0.061
Fe, µg/dl	84.62 ± 0.32	76.90 ± 0.44	0.472
Zn, µg/dl	44.44 ± 12.04	133.89 ± 27.64	0.017
Lipase, IU/L	2.62 ± 0.33	2.75 ± 0.60	0.857
Creatine kinase, IU/L	544.39 ± 97.58	426.28 ± 85.21	0.383
Alanine transaminase, IU/L	263.94 ± 35.56	285.84 ± 57.00	0.760
Aspartate aminotransferase, IU/L	290.71 ± 26.62	275.87 ± 36.90	0.758

*Blood samples were taken upon overnight fasting for 14 h at the age of 16 weeks. Each treatment group contained 16 males and four females.

growth such propagate disorders as hip dysplasia and osteochondrosis (21). Growth occurs through cell division and requires DNA, RNA and protein synthesis. Zinc as a cofactor for many enzymes influences gene expression through transcription factors (22). Growth hormone and IGF-I are the main hormones for growth, and these circulating hormones decreases with zinc deficiency in rats (10,11). The bones are primary targets for growth hormone which stimulates the secretion of IGF-I from the liver (23).

Specialized Zn transporters are involved in the regulation of intestinal zinc uptake, maintaining Zn homeostasis (24). Zinc transporter ZIP4 increases the concentration of intracellular zinc, while zinc transporter ZnT1 decreases the intracellular zinc level through export of zinc ions from the cytoplasm into the extracellular matrix (24,25). In a study with weaned pigs, Martin et al. (26) reported that excessive zinc uptake and a zinc-overload in the organism was maintained by up-regulation of ZnT1 and down-regulation of ZIP4 with the high dietary zinc supplementation. The same researchers also found that early zinc supplementation after weaning increased daily weight gain and feed in take, but the effect was reversed after three weeks of weaning. The results from the work of Martin et al. (26), along with those of present work may indicate that supplementing zinc positively influences the live performance of the animal in a short period of time, probably a couple of weeks only. Afterwards, the intestinal Zn absorption is subject to down-regulation, not being kept up with supporting growth and weight gain any longer. Only small increases of growth in puppies fed a Zn-supplemented diet of the present work could have been due to the mentioned mechanism (26).

Contrary to the results of the live weight changes and skeletal growth parameters, supplementing Zn to the diet of Labrador puppies influenced the serum parameters. Supplemental Zn did not influence the P but caused decreased serum concentrations of Ca and Mg. Not in agreement with the results of the present study, (12) found that Labrador puppies fed a diet containing either 50 or 200 ppm Zn for 140 days had similar plasma Ca concentrations.

Supplementing Zn to the diet of puppies resulted in increases in serum cholesterol and glucose concentrations. Contrary to the results of the present work, it has been found that supplementing Zn to the individuals had no effects on plasma cholesterol or triglyceride concentrations (27,28). Decreased serum concentrations of glucose at the present work were not expected because

Zn plays an important role in carbohydrate metabolisms. Stated that Zn supplementation in patients with diabetes has beneficial effects on glycemic control and promotes healthy lipid parameters (29). Zinc is involved in insulin metabolism, synthesis, storage, secretion, and signaling (30). Insulin secretion by glucose-stimulated pancreatic β cells is probably modulated by zinc's effect on the K_{ATP} channel in the signaling cascade (31). In achieving control of hyperglycemia in dogs with diabetes mellitus, insulin should be administrated daily. Found that using Zn as a complex with insulin (protamine zinc insulin, rhPZI) is effective in diabetic dogs and can be considered as an alternative treatment in diabetic dogs due to its long-acting feature (32).

Serum concentrations of Cu decreased upon dietary Zn supplementations in puppies. In parallel to the results of the present work, high plasma zinc concentrations have been reported to suppress copper uptake in dogs (33). In addition, Zn has been shown to induce metallothionein in hepatocytes, thereby binding free copper (34). Found that Labrador puppies fed a diet containing either 50 or 200 ppm Zn for 140 days had similar Fe and Cu plasma concentrations (12).

In a review, (35) stated that Zn supplementation to humans had no effects on iron-status indicators such as hemoglobin or serum ferritin. Iron serum concentrations were also remained similar between the groups of puppies at the present work. However, Zn supplementation in adults has been reported to decrease Fe absorption, hemoglobin, and serum ferritin concentrations (36,37).

As expected, supplementing Zn to the diet of puppies increased the serum concentrations of Zn. Also found that Labrador puppies fed a diet containing greater Zn supplementation (50 vs. 200 ppm) had greater plasma Zn concentrations (12).

CONCLUSIONS

The results of the present study indicated that supplementing 100 ppm zinc to the diet of Labrador puppies did not change weight gain but, although not greatly, promoted skeletal growth. Serum parameters of clinical importance were influenced by Zn supplementation. However, at this point, it is not recommended to supplement Zn more than already recommended level for dogs.

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