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Some macromineral and trace mineral levels in milk of different dog breeds

İnci Söğütlü[1] Nizamettin Günbatar[2] Handan Mert[3] Mustafa Cellat[4] Nihat Mert[3]

[1] *Department of Food Establishments and Codex Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkey*
[2] *Health Sciences Faculty, Van Yuzuncu Yil University, Van, Turkey*
[3] *Department of Biochemistry, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey*
[4] *Department of Physiology, Faculty of Veterinary Medicine, Mustafa Kemal University, Antakya, Turkey*

Correspondence: *İnci Söğütlü (incidoganbiyo@hotmail.com)*

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ABSTRACT

Objective: In this study, it was planned to examine the mineral levels in the milk of different dog breeds during the lactation period. Calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) concentrations were analyzed in dog milk.

Materials and methods: In this research 6 Labradors, 6 German Shepherds, 6 Pointers, 5 Turkish Tazis (sighthound), 5 Setters, 7 Malinois, and 5 Golden Retrievers (a total of 40 dogs) of 3 to 4 years of age were used as research materials, all of which were under same management and feeding conditions. All dogs were on diets appropriate for gestation and lactation periods. Adequate milk volume could be collected 2-3 weeks after parturition, and there were no known medical problems. Each day's samples were kept capped and refrigerated after being collected. The concentrations of calcium, magnesium, potassium, sodium, copper, zinc, manganese and iron, were analyzed by using Varian Brand 30/40 model AAS device.

Results: The Ca, K, Na, Zn, Mn and Fe levels of milk samples from different dog breeds had no significant difference. The highest Mg level was determined in Pointer breed milk samples, and the lowest was determined in Setter milk samples. The highest Cu levels among the inspected races were in Labrador milk samples, whereas the lowest levels were determined in Setter breeds.

Conclusion: This data shows that most of the analyzed milk content of different breeds of dogs did not change significantly during the same lactation period, and any present difference could be taken into account when evaluating breeding studies.

Keywords: *Dog, milk, Macro minerals, Trace minerals*

INTRODUCTION

Dog milk is a fluid that contains enough nutrients that are secreted at different times in the milk glands to feed newborn puppies of female dogs, and the puppy is obliged to consume until it is able to feed itself (Ergun and Mert, 1984). Milk is regarded as a basic food substance by nutritionists. It is important for its calcium, phosphorus, and riboflavin (Vit B2) content. It also contains essential amino acids and fatty acids. Certain substances are found only in milk, like lactose, casein, lactalbumin, and lactoglobulin. The energy value of milk differs based on its composition. One liter of 3% fat consumer milk provides 615 kcal energy. The composition of the milk differs based on the environmental conditions in which the lactating animal lives. Dogs reach twice their birth weight in 9 days, perhaps related to the protein content of the dog's milk, which is 7.3%. Humans, on the other hand, reach twice their birth rate within 180 days, and the protein content of human milk is 1.6% (Bremel, 1995).

Some major and trace elements are necessary for mammals in many physiological functions such as bone and cartilage formation, enzymatic reactions, intracellular and extracellular fluid balances, oxygen transport, electron transfer reactions, normal muscle and nerve functions and hormone production. It is reported that imbalances arising from excess minerals or their deficiencies are associated with pathological conditions (Mert et al., 2008).

Na, K, Ca, Mg, Cl, and phosphate are the basic mineral substances in milk, and there are also many trace elements in it (Ergun and Mert, 1984). The trace elements in the milk are mostly ionic and in salt forms. Since their quantities are very small, their presences are investigated spectrometrically. Generally, those with a lower presence than that can be expressed with "mg/kg" are regarded as trace elements. Feeding of the mother affects the amount of trace element levels. The amount may also vary according to the point of time during the lactation period. In contaminations, some metals may pass through the milk and can change the actual amount. Some trace elements have vital preservation roles and are called micronutrient elements. Micronutrients are Iron, Copper, Cobalt, Zinc, and Iodine. Micronutrients are important in nutritional physiology, such as the cobalt taking part in vitamin B12 structure. They are found in the composition of enzymes, and Fe is involved in the structure of catalase and peroxidase enzymes. They may act as enzyme activators or inhibitors as well.

They also take part in certain chemical reactions that take place within milk and milk products, which may cause quality defects. Cu, for example, causes autooxidation of milk fat. The trace elements are found in the coenzymes (prosthetic group) of the enzymes in the milk. Molybdenum, for instance, is involved in the structure of the xanthine oxidase enzyme. They are also influential in the activity of enzymes. For example, Mg^{++}, Mn^{++}, Co^{++}, and Zn^{++} enhance the activity of alkaline phosphatase. When the amount of trace elements increases, they play a catalytic role in chemical and biochemical reactions and have an adverse effect in high quantities. An increase in copper (>10-5 mol) leads to ascorbic acid autooxidation in milk. For this reason, tools and equipment made of copper shouldn't be used (Keen et al., 1982; Bremel, 1995).

In this study, it was aimed to determine the mineral levels in milk samples obtained from different dog breeds.

MATERIALS and METHODS

The study was conducted according to ethical guidelines and under the supervision of Hatay Mustafa Kemal University Local Ethics Committee board (*Decision no: 2022/01-11*). All animal-use protocols were carried out in accordance with Directive 2010/63/EU of the European Parliament and Council of 22 September 2010 on the protection of animals used for scientific purposes (European Union Directive, 2010). In this research 6 Labradors, 6 German Shepherds, 6 Pointers, 5 Turkish Tazis, 5 Setters, 7 Malinois, and 5 Golden Retrievers (a total of 40 dogs) of 3 to 4 years of age were used as research materials, all of which were under the same management and feeding conditions. Depending on the age of the animals and the orientation of the dogs, commercial dog food was used and an individualized feeding program was applied. The composition of this commercial dog food was as follows: crude protein minimum 28%, crude fat minimum 16%, crude cellulose maximum 3% and crude ash maximum 7%. Additionally, all dogs were on diets appropriate for gestation and lactation periods. Adequate milk volume could be collected 2-3 weeks after parturition, and there were no known medical problems. Each day's samples were kept capped and refrigerated after being collected. Within 24 hours after collection of the last day's sample, all samples were transported to the laboratory in a cold chain. Samples were then warmed (+5°C) in a heat block and turned over repeatedly to resuspend any cream layer that had separated. Maximum attention was given to prevent contamination of the mineral matter of the samples (Salisbury and Chan, 1985).

Homogenized milk samples were digested in acid-rinsed glass Pyrex tubes using trace metal–grade concentrated nitric acid for 5 h at 125°C for AAS measurements. The concentrations of calcium, magnesium, potassium, sodium, copper, zinc, manganese, and iron were investigated in dog milk samples during the course of the lactation period. Mineral analyses were performed using Varian Brand 30/40 model AAS device and the Varian GTA-96 graphite tube atomizer with electrothermal atomization method. The measurements were made in automated mode and were repeated twice (Tayar et al., 1993; Mert et al., 1994).

Statistical analysis

SPSS 22.0 Windows program (evaluation version) was used for statistical analysis of the data. One-way ANOVA test was used for data analysis; Duncan test was used for multiple comparison tests. P<0.05 was considered statistically significant.

RESULTS

The mineral levels of milk samples obtained from different dog breeds are given in Table 1. Ca, K, Na, Zn, Mn and Fe levels were found to have no significant difference among breeds. The highest Mg level was found in Pointer milk samples and the lowest was in Setter milk samples (p<0.001). The highest Cu levels amongst races were found in Labrador (5.14±0.94), Turkish Tazi (5.04±0.48) and German Shepherd (4.98±0.66) breed milk samples, while the lowest levels were determined in Setter (3.97±0.35) and Golden Retriever (4.11±0.66) samples (p<0.001).

Table 1. Some macro and trace minerals levels in milk samples obtained from different dog breeds.

Parameters (mmol/L)	X±Sx							p Value
	Labrador (n=6)	German Shepherd (n=6)	Pointer (n=6)	Turkish Tazi (n=5)	Setter (n=5)	Malinois (n=7)	Golden Retriever (n=5)	
Ca	5.85±1.18	5.03±0.98	4.46±0.77	6.17±1.22	5.45±1.02	5.88±0.98	4.95±0.77	0.073
Mg	0.87±0.11[a]	0.71±0.09[bc]	0.89±0.13[a]	0.77±0.09[abc]	0.65±0.08[c]	0.69±0.07[bc]	0.81±0.08[ab]	0.001
K	12.65±1.74	10.44±1.44	11.66±1.34	11.55±1.26	12.01±1.08	11.79±1.11	12.46±1.36	0.160
Na	5.02±1.07	4.74±0.99	4.98±0.98	4.56±0.77	4.98±0.67	4.95±1.08	5.13±1.05	0.972
Cu	5.14±0.94[a]	4.98±0.66[a]	4.46±0.75[ab]	5.04±0.48[a]	3.97±0.35[b]	4.76±0.45[ab]	4.11±0.66[b]	0.027
Zn	44.19±4.64	43.72±3.98	47.18±4.08	41.76±3.65	42.68±3.99	40.92±4.06	46.78±4.14	0.099
Mn	0.18±0.04	0.15±0.03	0.23±0.05	0.20±0.02	0.22±0.06	0.19±0.04	0.18±0.03	0.053
Fe	6.63±1.21	6.88±1.32	7.04±1.34	6.91±1.04	6.42±1.03	6.76±1.12	7.10±1.12	0.969

DISCUSSION

It is important to know which trace elements are required for adequate growth and development during this nutritionally challenging life expectancy since infants usually receive all their nutrition from one type of food. A number of factors affect the trace element content of the milk (Davidson et al., 1994).

Heinze et al. (2014) analyzed the mineral content of homogenized breast-milk in samples digested with concentrated nitric acid (70%), and found the levels of some minerals as; calcium 6.56±1.22 mmol/L, magnesium 0.93±0.18 mmol/L, potassium 15.1±2.1 mmol/L, sodium 6.35±1.4 mmol/L and copper 9.24±1.81 μmol/L, iron 10.0±7.9 μmol/L, manganese 0.21±0.15 μmol/L, rubidium 13.1±2.9 μmol/L, selenium 0.21±0.06 μmol/L, strontium 0.50±0.16 μmol/L, Zinc 66.7±14.4 μmol/L.

Microminerals also called micronutrients or trace elements, have been described physiologically as substances that are less than 0.01% of body weight (Ergun and Mert, 1984; Mert et al., 1993). This term covers all elements, except for those that make up the organic matrix (carbon, hydrogen, nitrogen, oxygen, and sulfur), as well as body minerals of biological fluids and skeletons (calcium, magnesium, potassium, sodium, chlorine and phosphorus). Iron is the boundary between macroscopic and microminerals and is often processed separately because of its long history and well-documented physiology (Davidson et al., 1994).

Concrete clinical syndromes associated with deficiency of zinc, copper, and iodine are well described (Casey and Walravens, 1988). Iron deficiency does not appear in infants in humans before 6 months due to large deposits of iron at birth (Cavell and Widdowson, 1964; Dallman, 1988). Although specific physiological and/or enzymatic functions can be attributed to molybdenum and manganese, nutritional deficiencies in these elements have not been documented in infants (Casey and Walravens, 1988). The bioavailability of a large number of trace elements that are considered important or important for the growth of a child is available only for iron, zinc, copper, manganese, and selenium.

The concentration of most trace elements in human milk is less dependent on the consumption of the mother or blood (IOM, 1991). Exceptions are "anionic" elements: iodine, fluorine, and selenium. Excessive consumption of many elements, especially those that are metabolized as anions, can be associated with a risk of toxicity for the infant, and strengthening of the suckling mother in terms of these elements is usually not recommended.

The concentration of iron in the milk in most of the domestic animals are in the range of 0.2-1.0 pg/mL at slightly higher levels than in the colostrums. Lonnerdal et al. (1982) reported that about 20% of iron was found to have fat content in the milk and 30-60% in the casein fraction. Iron levels in other animals are as follows: cows 0.2-0.6 μg/mL, buffalo 0.2-0.3 μg/ml, goat 0.3-0.4 μg/mL (Lonnerdal et al., 1981; Kincaid and Cronrath, 1992).

Zinc is an important component of more than 200 enzymes that can act both catalytically and structurally (Hambidge et al., 1986; IOM, 1991). It seems that it plays a critical role in the expression of genes as well: many DNA-binding proteins are zinc complexes. Zinc metalloproteins are also important for protecting the integrity of cell membranes and extracellular matrix architecture (Waxman and Wasan, 1992). Depending on the extent of zinc depletion, deficits in young mammals can lead to delayed growth, anorexia and severe diarrhea and skin lesions (Hambidge et al., 1986; Casey and Walravens, 1988). Due to higher growth rates in men and premature babies, the demand for zinc is increased and they are more vulnerable to deficits (Krebs and Hambidge, 1986). Zinc is found in three main fractions of milk; fat, casein and whey, in various chemical forms. The concentration of zinc is higher in colostrum, which decreases during lactation. Zn concentrations in dogs ranged from 7 to 8 (μg/mL) according to Anderson et al. (1991) and Lonnerdal et al. (1981) whereas in cats it is 5-7 (μg/mL) according to Keen et al. (1982).

Copper is a component of many metalloenzymes such as cytochrome oxidase, superoxide dismutase, ceruloplasmin, enzymes that play a role in the transport of copper into tissues and in the release of iron and in the synthesis of connective tissue, melanin, and catecholamines (Casey and Walravens, 1988). The deficiency of copper as a result of malnutrition is observed in humans and other species, especially amongst young members. Interaction with excess molybdenum and sulfate may be important in the etiology of copper deficiency in pastoral animals. In general, insufficiency in young mammals causes anemia, anorexia, diarrhea, bone disorders, and defects in cartilage, hair growth, and pigmentation (Davis and Mertz, 1987). In many newborns, including humans, the concentration of copper in the liver is higher than in adults, except for sheep. In humans and mice, the level of fetal liver copper can reach up to 10 times in comparison with adults. This copper is closely related to intracellular metallothionein. In all species, the levels in the colostrum are higher but are reduced after a period of lactation. The drop in the copper amount in milk is usually less than the drop in the zinc level, however, a significantly higher percentage, 30-40% of copper in animal milk, is found associated with casein, compared to human milk. In companion animals, the levels are usually an order of magnitude higher than in other animals. In canine milk, the concentration of Cu is 1.3-2 (μg/mL) according to Anderson (1991) and Lonnerdal et al., (1981). In cats, the Cu amount is 0.8-1.2 (μg/mL) according to Keen et al (1982).

Metalloenzymes of manganese have a wide range of metabolic functions: synthesis of mucopolysaccharides, gluconeogenesis, lipid metabolism, neurotransmitters and synthesis of mitochondrial superoxide dismutase at the same time. Manganese deficiencies have been obtained experimentally in several species, but are naturally found in pig and poultry diets, yet have never been found in some people (Hurley and Keen, 1987). Fetal life and premature infancy are the most sensitive periods of manganese deficiency. In dogs, the Mn amount in milk is about 140 (ng/mL), but in colostrum, it was measured approximately 160 (ng/mL) by Lonnerdal et al. (1981).

The main ionic constituents of milk are monovalent ions of sodium, potassium and chloride, and bivalent calcium, magnesium, citrate, phosphate and sulfate ions. Monovalent ions of sodium, potassium, and chloride are amongst the most prevalent minerals in milk, collectively contributing 30 mOsm or a tenth of the total osmolarity of human milk, 82 mOsm or a quarter of the osmolarity of bovine milk, and 196 mOsm or almost two-thirds of the osmolarity of rabbit milk

(Peaker, 1977). Significant changes in concentrations of basic monovalent cations in milk are associated with conditions that facilitate the close bonds between epithelial cells. The main pathological process that changes the content of a monovalent cation in milk is mastitis or localized breast tissue inflammation. Inflammation opens connections between cells, and changes in sodium and chloride content can be determined by measuring the electrical conductivity of milk from animals with mastitis (Linzell and Peaker, 1971). In general, changes in concentration are not associated with systemic diseases, such as diabetes (Butte et al., 1987), cystic fibrosis or local diseases such as mastitis. Some effects on calcium and magnesium have been observed by some authors (Lonnerdal, 1986a; Lonnerdal, 1986b). There are few reports that claim pharmacological doses of magnesium sulfate increase the magnesium concentration in colostrum (Cruikshank et al., 1982). Dietary and seasonal effects on the concentration of calcium and citrate in bovine milk have been reported by Halt and Muir (1979). As the serum citrate increases, calcium increases markedly in the first few days after birth, and then slowly drops to 6.6 mmol/L in the first 3 months after birth. After 3 months, calcium level slowly and consistently falls. Iron, zinc, copper, and magnesium concentrations decrease during lactation, whereas calcium and phosphorus concentrations increase.

During the first 45 days of lactation, iron, copper, zinc, manganese, calcium, magnesium, protein, carbohydrate and fat content of dogs were analyzed. The concentration of iron significantly decreased from 13 µg/mL to 0 to 6 µg/mL. The concentration of zinc is reduced from 9.6 µg/ml to 8.7 µg/mL. During early lactation, the calcium concentration increased from 1.366 µg/mL to 1.757 µg/ml on day 10 but then changed slightly. Concentrations of copper, manganese, magnesium, and carbohydrates did not show strong developmental structures; the mean values were 1.8 µg/mL, 0.14 µg/mL, 59 µg/mL and 4.5%, respectively (Lonnerdal et al., 1981).

CONCLUSION

The concentrations of calcium, magnesium, potassium, sodium, copper, zinc, manganese and iron were analyzed in dog milk during the course of lactation. The Ca, K, Na, Zn, Mn and Fe levels of milk samples from different dog breeds were found to have no significant difference. The highest Mg levels were determined in Pointer milk samples and the lowest levels were found in Setter milk samples. The highest Cu levels amongst races were found in Labrador milk samples whereas the lowest levels were determined in Setters. These data show that milk mineral content of different breeds of dogs should be taken into account in nutritional studies.

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REFERENCES

Abram Anderson RS, Carlos GM, Robinson IP, *et al.* Zinc, copper, iron and calcium concentrations in bitch milk. J Nutr. 1991; 121(11):81-82.

Bremel RD. Handbook of milk composition. 1st ed. Cambridge: Academic Press; 1995.

Butte NF, Garza C, Burr R, *et al.* Milk composition of insulin-dependent diabetic women. JPGN. 1987; 6(6):936-941

Casey CE, Walravens PA. Trace elements. In: Tsang RC, Nichols BL, eds. Nutrition During Infancy. Philadelphia: Hanley & Belfus; 1988. p.190-215.

Cavell PA, Widdowson EM. Intakes and excretions of iron, copper, and zinc in the neonatal period. Arch Dis Child. 1964; 39(207):496-501.

Cruikshank DP, Varner MW, Pitkin RM. Breast milk magnesium and calcium concentrations following magnesium sulfate treatment. Am J Obstet Gynecol. 1982; 143(6):685-688.

Dallman PR. Nutritional anemia of infancy: iron, folic acid, and vitamin BI2. In: Tsang RC, Nichols BL, eds. Nutrition During Infancy. Philadelphia: Hanley and Belfus; 1988. p.216-235.

Davidson L, Kastenmayer P, Yuen M, Lonnerdal B, Hurrell R. Influence of lactoferrin on iron absorption from human milk in infants. Pediatr Res. 1994; 35(1):117-124.

Davis GK, Mertz W. Copper. In: Mertz W, eds. Trace Elements in Human and Animal Nutrition. San Diego: Academic Press; 1987. p.301-364.

Ergun H, Mert N. Biochemical changes due to mastitis in milk. I. Mastitis Seminar. Ankara Univ Vet Fak. 1984; 46-61.

European Union Directive. Directive 2010/63/EU of the European Parliament and of the Council of the protection of animals used fer scientifıc purposes. Official Journal of the European Union. 2010; 276:33-79.

Halt C, Muir DD. Inorganic constituents of milk: I. Correlation of soluble calcium with citrate in bovine milk. J Dairy Res. 1979; 46(3):433-439.

Hambidge KM, Casey CE, Krebs NF. Zinc. In: Mertz W, eds. Trace Elements in Human and Animal Nutrition. 5th ed. Orlando: Academic Press; 1986. p.1-138.

Heinze CR, Freeman LM, Martin CR, *et al.* Comparison of the nutrient composition of commercial dog milk replacers with that of dog milk. J Am Vet Med Assoc. 2014; 244(12):1413-1422.

Hurley LS, Keen CL. Manganese. In: Mertz W, eds. Trace Elements in Human and Animal Nutrition. 5th ed. San Diego: Academic Press; 1987. p.185-223.

Institute of Medicine (IOM). Nutrition during lactation. Subcommittee on Lactation., Committee on Nutritional Status during Pregnancy and Lactation, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. Washington: National Academy Press; 1991.

Keen CL, Lonnerdal B, Clegg MS, *et al.* Developmental changes in composition of cats' milk: Trace elements, minerals, protein, carbohydrate and fat. J Nutr. 1982; 112(9):1763-1769.

Kincaid RL, Cronrath JD. Zinc concentration and distribution in mammary secretions of peripartum cows. J Dairy Sci. 1992; 75(2):481-484.

Krebs NF, Hambidge KM. Zinc requirements and intakes of breast-fed infants. Am J Clin Nutr. 1986; 43(2):288-292.

Linzell JL, Peaker M. Early detection of mastitis. Vet Rec. 1971; 89:393-394.

Lonnerdal B, Keen CL, Hurley LS, Fisher GL. Developmental changes in the composition of Beagle dog milk. Am J Vet Res. 1981; 42(4):662-666.

Lonnerdal B, Keen CL, Hurley LS. Trace elements in milk from various species. In: Howell McC, Hawthorne JM, White CL, eds. Trace Element Metabolism in Man and Animals, TEMA-4. South Australia: Netley, Griffen Press; 1982. p.249-252.

Lonnerdal B. Nutrition and lactation. In: Hamosh M, Goldman AS, eds. Human Lactation 2: Maternal and Environmental Factors. New York: Plenum Press; 1986a.

Lonnerdal B. Effects of maternal dietary intake on human milk composition. J Nutr. 1986b; 116(4):499-513.

Mert H, Mert N, Doğan İ, Cellat M, Yaşar S. Element status in different breeds of dogs. Biol Trace Elem Res. 2008; 125(2):154-159.

Mert N, Tayar, M, Şen C, *et al.* Determination of Lead (Pb) levels in milk consumed in Bursa region. EU Faculty of Sci. 1994; 12(1):173-176.

Salisbury CD, Chan W. Simple automated wet digestion of animal tissues for determination of seven elements by atomic absorption spectroscopy. J Assoc Off Anal Chem. 1985; 68(2):218-219.

Peaker M. The aqueous phase of milk: Ion and water transport. Symp Zool Soc Lond. 1977; 41:113-134.

Tayar M, Mert N, Çetin M, *et al.* Liver lead levels of slaughtered cattle in Bursa. U U Veteriner Fak Dergisi. 1993; 16(1):16-20.

Waxman J, Wasan H. The architecture of cancer. Br Med J. 1992; 305:1306-1307.