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RESEARCH ARTICLE

The Effects of Pregnancy, Early Lactation Weeks and the Number of Suckling Kittens on the Serum Mineral and Metabolite Profiles of Van Cats in Breeding Season

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Summary

In Van cats, changes in serum mineral (Na-Sodium, K-Potassium, Ca-Calcium, P-Phosphorus, Mg-Magnesium, Cl-Chlorine and Fe-Iron) and metabolite concentrations (TG-Triglycerides, COL-Cholesterol, T.Prot.-Total protein and ALP-Alkaline phosphatase) were investigated before, during and after the pregnancy. A total of 22 mature cats (19 queens and 3 fertility-proven tomcats) were used as material. Thereafter, 13 queens showed oestrus while others (n=6) did not. Samples of venous blood were collected from anoestrous and oestrous queens upon hand-mating, on the 54th day. After parturition (n=7), the number of live kittens was recorded and additional samples were collected on the 5th, 12th and 19th days of lactation. Results were also considered for small (n=3) and large number (n=4) of suckling kittens (≤4 or >4 litters, resp.). For minerals, there was a significant ($P<0.01$) fluctuation in K levels during the prolonged lactation with large number of suckling kittens. However, significant ($P<0.05$) decrease was found for P value as the lactation period prolonged. Meanwhile, for metabolites, TG levels in previously-mated queens (becoming pregnant or not) were significantly ($P<0.01$) higher than those both in anoestrus and in lactation. Also, COL levels in pregnant queens were significantly ($P<0.05$) higher than those in all other occasions and further that there were significant decreases in COL levels both in the first and the second weeks of lactation. Collectively, present findings suggest that; a) the concentrations of K and P minerals and metabolites studied *per se* (TG, COL, T.Prot. and ALP) changed markedly during the late pregnancy and prolonged lactation, and b) declines in minerals (P) and metabolites (COL) become even greater when the number of suckling kittens increased in queen Van cats during breeding season.

Keywords: *Van cat, Pregnancy, Lactation, Litter size, Mineral, Metabolite*

Üreme Sezonundaki Van Kedilerinde Gebelik, Erken Laktasyon Haftaları ve Emzirilen Yavru Sayısının Serum Mineral ve Metabolit Düzeyleri Üzerine Etkileri

Özet

Van kedilerinde, gebelik öncesi, esnası ve sonrasında serum mineral (Na-Sodyum, K-Potasyum, Ca-Kalsiyum, P-Fosfor, Mg-Magnezyum, Cl-Klor ve Fe-Demir) ve metabolit konsantrasyonlarındaki (TG-Trigliserid, COL-Kolesterol, T.Prot.-Total protein ve ALP-Alkalin fosfataz) değişiklikler araştırıldı. Materyal olarak, toplam 22 adet yetişkin kedi (19 dişi ve 3 fertil erkek) kullanıldı. Dişilerin 13 tanesi sonradan östrüs gösterirken, 6 tanesi hiç bir belirti göstermedi (anöstrus). Hem anöstrüsteki hem de elde-çiftleştirme sonrası, 54. günde, gebe kalmayan (n=6) ve kalan dişilerden (n=7) venöz kan örnekleri alındı. Doğum sonrası canlı yavruların sayısı kaydedildi ve laktasyonun 5., 12. ve 19. günlerinde emziren dişilerden kan örnekleri alındı. Ayrıca, sonuçlar bir batında doğan az (n=3) veya çok (n=4) sayıdaki emzirilen yavrular (sırasıyla ≤4 veya >4 adet yavru sayısı) yönünden değerlendirildi. Elde edilen bulgulara göre, mineraller yönünden, özellikle uzun laktasyon döneminde çok sayıda yavru emziren kedilerdeki K düzeylerinde önemli ($P<0.01$) dalgalanma gözlendi. Ancak, emzirme döneminde P değerinde önemli ($P<0.05$) bir azalma vardı. Öte yandan, metabolitler yönünden çiftleşme sonrası (gebe kalmayan veya kalan) kedilerdeki TG düzeylerinin, hem anöstrustaki hem de laktasyondakilerden anlamlı düzeyde ($P<0.01$) daha yüksek olduğu bulundu. Ayrıca, gebe kedilerdeki COL düzeylerinin diğer tüm durumlardaki kedilerden önemli oranda ($P<0.05$) daha yüksek olduğu gözlenmekle birlikte, laktasyonun hem ilk hem de ikinci haftasındaki COL düzeylerinde önemli azalma vardı. Sonuç olarak, üreme sezonundaki dişi Van kedilerinde; a) incelenen minerallerden K ve P'nin, metabolitlerin ise tamamının (TG, COL, T.Prot. ve ALP) gebeliğin sonunda ve laktasyonun ilk haftalarında belirgin oranda değiştiği, ve b) mineral (P) ve metabolit (COL) düzeylerindeki azalmanın emzirilen yavru sayısının artmasıyla daha da belirginleştiği kanısına varıldı.

Anahtar sözcükler: *Van kedisi, Gebelik, Laktasyon, Yavru sayısı, Mineral, Metabolit*

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INTRODUCTION

Van cat is an endemic breed of Van province (in Eastern Anatolia, Turkey), has colourful eyes (one turquoise blue the other amber yellow, *Fig. 1*) and long/short white fur *(Fig. 2)* [1]. In general, cats are seasonally polyoestrous and the ovulation occurs after coital stimulation. Queens reach puberty, as evidenced by their first oestrus signs, between 7-12 months, depending on their parturition season, diseases and feeding conditions. Body weight has also a major influence such that queens show their first oestrus when they reach to 2.3-3.5 kg. Regarding parturition season *(Fig. 3)*, female kittens *(Fig. 4)* born during winter reach puberty in the first breeding season of the same year of age, while others born in spring do not show any signs until the next spring [2]. However, when the litter size decrease the body size of kittens born becomes greater. Also, the congenital abnormalities and the incidence of abortus increase as the queens become older [2,3]. In queens, pregnancy lasts about 65 days depending on age, breed

Fig 1. Young (2 years old) queen Van cat with eyes in different colours
Şekil 1. Farklı göz renklerine sahip genç (2 yaşında) dişi Van kedisi

Fig 2. Mature (4 years old) queen Van cat with white fur
Şekil 2. Beyaz tüylü yetişkin (4 yaşında) dişi Van kedisi

Fig 3. Mother queen suckling 3 kittens
Şekil 3. Üç yavru emziren anne kedi

Fig 4. Weaned (3 months old) female kittens
Şekil 4. Sütten kesilmiş (3 aylık) yavru kediler

and feeding. Multiple kitten-bearing pregnancies last from days 58 to 69 post-mating. It was reported that queens give a birth to 2-8 kittens *(Fig. 3)* along with lactation period enduring for about 2-3 months [2].

Elements available within the body can be classified as macro- and microelements. Amongst them, in one hand, the Ca, P, Na, K and Mg are all macroelements, available at milligram (mg) levels. On the other hand, the Cl, Fe Zn, Mn and Cu are at μg levels and are classified as trace elements [4]. These elements with their tiny amounts (less than 4 mg in total) available are essential substances required for biological functions. These elements are co-factors of numerous enzymes playing crucial roles in enzymatic reactions [5]. Not only the elemental deficiency but also the proportional alterations between trace elements may lead to physiological changes/disorders [6,7]. The majority of trace elements take part in the syntheses of enzymes, hormones and vitamins [8]. Among them, Fe and other metallic ions all enable the non-enzymatic lipid peroxidations [9]. Further, Fe plays an effective role mainly in oxygen transfer-storage (through haemoglobin) and in enzyme activation [10]. Hepatic Fe store is important for the first 6 months of a newborn, since the amount available in milk is low. Luckily, in colostrum, Fe concentration is 3-5 folds higher than that of the normal milk; hence, colostrum should be given to newborn, whenever available. Trace element deficiencies lead to clinical symptoms, such as; diarrhoea, anaemia, loosing hair (poor feathering), depigmentation, bone disorders, parakeratosis, loose appetite (deprivation), infertility, poor development of offsprings (low feeding efficiency), poor semen quality, tetanus, non-infectious abortus and pica [8,11]. Furthermore, these deficiencies may also adversely influence the protein synthesis [7,12,13]. Indeed, Fe available in milk is in protein-bound form, so-called as lactoferrin [14].

The metabolites of COL, TG and milk yield (milk with 3-4% fat minimum) are positively correlated together [15]. In cows treated with bST (bovine somatotropin), the elevated ALP activity is likely lead to increased mobilisation from the bones. Plasma glucose and COL concentrations could be affected adversely during the first days of oestrus behaviours. COL involved in steroid hormones has critical roles during pregnancy for partly justifying the energy requirement [16]. Also, glucose and TGs have positive correlation since they are inter-related with the energy requirement during the body weight loss. These clearly indicate that reproductive performance and energy requirement are not dependent upon a specific substance, but rather they are inter-linked with the level of numerous substances that have many functions in the general metabolism [17]. Excess T.Prot. in serum was reported to affect the fertility parameters unfavourably. But, there are some reasons stimulating this increase, e.g. excessive amount of protein intake along with the nutrients and/or the presences of protein structures such as urea and albumin [18]. Excessive intake by feeding can lead alkalosis, disruptive energy metabolism and liver damage in ruminants [19]. This condition could also lead to reproductive disorders such that the interval of calving-to-conception may be prolonged. Further, it could increase the blood urea level, resulting in the impairment of sperm transport within the genital tract prior to fertilisation [20].

Kittens of domestic cats require mothers' milk at least until the 4 weeks of age. During the 1-3 weeks of lactation, kittens gain 150-350 g live weight. At the end of 5 weeks, they begin to eat small amounts of other feed and are eventually weaned at 8-10 weeks. Hence, mineral requirement of suckling queens increases during the lactation period. In lactating cats, the intake of Ca, P, Mg, Zn and Mn increases, while that of the Fe and Cu remains stable [21]. Elsewhere, Keen et al.[22] and Adkins et al.[6] previously given the composition of minerals in milk produced.

In the literature, most of the previous reproduction studies related with the minerals and metabolites were carried out in dairy cows [23,24], due presumably to their huge economic importance and nutritional (protein) values across the globe. In this area of research, although there may also be some other reports in other species (namely small ruminants), but the studies in queens are rather scarce. Furthermore, scientific studies in endemic Van cats are limited. Therefore, the objectives of the present study were to investigate firstly; i) the initial serum mineral (Na, K, Ca, P, Mg, Cl and Fe) and metabolite levels (TG, COL, T.Prot. and ALP) of anoestrous, non-pregnant (post-mating) and pregnant queens, ii) monitore the changes after parturition (during the first three weeks of lactation) and, finally iii) the effect of suckling kitten numbers upon the mineral and metabolite levels in blood in lactating Van cats during breeding season.

MATERIAL and METHODS

Animals and Their Care

The study was conducted during the breeding season (mid-January onwards), using 22 queen Van cats and 3 tomcats (known to be fertile) at the Van Cat Research Centre of Yuzuncu Yil University, Van-TR. The age of the cats ranged from 3 to 9 years old. The criteria for selection of cats were as follows: Queens were selected according to their maturity and mating history, while tomcats were selected considering their sufficient/ongoing libido and history of proven fertility in the previous mating season. All the vaccinations and antiparasitic drug administrations of queens kept in the cat shelter have being made routinely.

In the Van Cat Centre, animals have being kept in different management rooms for; i) mature queens, ii) mature tomcats, iii) kittens, iv) mating, v) parturition and vi) quarantine. Cats were fed by a standard commercial diet (La Cat®, Israel) with water *ad libitum* throughout the year. Certified nutrient levels and the ingredients of commercial cat diet used (La Cat®, Israel) are illustrated in *Table 1*.

Table 1. *Certified nutrient levels and the ingredients of commercial cat diet used (La Cat®, Israel)*

Tablo 1. *Kullanılan ticari kedi mamasının (La Cat®, Israel) sertifikalı besin düzeyleri ve içerikleri*

Parameter Analysed	Percentage (of 100 g)
Protein	30
Fat	13
Fibre	2.5
Ash	6.8
Moisture (max.)	10
Sodium	0.6
Phosphorus	1.0
Calcium	1.1
Magnesium	0.1
Metabolic energy (kCal/kg)	3.600

Ingredients: *chicken flour, chicken liver flour, fish flour, corn, wheat, corn gluten flour, chicken fat (stabilised by BHT, as antioxidant), beer yeast, natural flavouring extracts, salt, vitamins, minerals and trace elements, taurine, yucca*

Blood Collection

Blood samples were collected routinely from *V. cephalica antebrachii*, using Gr. 0.80 x 78 mm cannula into 5 ml serum tubes. Sera were separated by centrifugation at 3.000 *g* for 10 min. Then, the samples in eppendorf tubes (1.5 ml) were kept at −20°C until the analyses.

Experimental Design

During the breeding season, oestrous queens were transferred into the mating rooms. Following the matings, the presences of pregnancy were detected by both inspection and abdominal palpation [25] on the 54th day post-mating. On that day, the first blood samples were collected. Next, animals were divided into two experimental groups, as; i) non-pregnant (post-mating), as control (n=6, Group I), and ii) pregnant queens (n=7, Group II). Following parturitions, mother queens were further allocated into two further subgroups, as small (≤4, n=3 litters) or large (>4, n=4 litters) number of suckling kittens. Further samples of blood were collected three times on the first (the 5th d), second (12th d) and third weeks (19th d) of lactations. No further samples were collected from non-pregnant ones.

Laboratory Analysis

Serum Na, K, Ca, P, Mg, Cl (mmol/ml, all) and Fe (μmol/ml) concentrations from control (anoestrous and/or non-pregnant) and pregnant groups were measured at the laboratories of Justus-Liebig University (Giessen, GERMANY). All the mineral levels were assessed using an ion-selective electrode of a Radiometer (ABL® SYSTEM 615, Copenhagen, DENMARK). Additionally, serum metabolite levels of TG (mmol/l) and ALP (U/l) were determined by the photometric GDO-PAP method (GPO-PAP Flüssig®, Labor + Technik Eberhard Lehmann). Further, COL concentrations (mmol/l) were measured by the enzymatic colour test using CHOD-PAP method (Labor + Technik Eberhard Lehmann). Finally, T.Prot. levels (g/l) were determined by manual refractometer using HRM 18 (Krüss).

Statistical Analysis

Data from the mineral (Na, K, Ca, P, Mg, Cl and Fe) and metabolite levels (TG, COL, T.Prot. and ALP) during anoestrus, before and after pregnancy, lactation period and the number of suckling kittens were presented as mean ± SEM. The values from anoestrus, different pregnancy statues (+/-), lactation period (the first, second and third weeks) and the number (small and large) of suckling kittens were analysed by Pearson's correlation and regression analysis using MINITAB statistical software programme [26]. Differences between the groups and subgroups were considered significant, using the least significant differences (when P≤0.05).

RESULTS

Mineral Profiles

Unlike other minerals (Na, K, Ca, Mg, Cl and Fe) remaining statistically (virtually) unchanged, there was significant (P<0.05) decrease for P value as the lactation period prolonged *(Table 2)*. However, a significant (P<0.01) fluctuation in K levels was found during the prolonged lactation with large number of suckling kittens *(Table 3)*. Nevertheless, P levels were either significantly (P<0.01) decreased in lactating queens with small number of suckling kittens or were fluctuated in queens with large number of kittens.

Metabolite Profiles

TG levels in non-pregnant/pregnant queens were significantly (P<0.01) higher than those both in anoestrus

Table 2. *Effects of pregnancy and lactation stage upon the serum mineral status of Van cats during breeding season*
Tablo 2. *Gebelik ve laktasyon sürecinin üreme sezonundaki Van kedilerinde serum mineral düzeyi üzerine etkileri*

*Minerals	Physiological factors						Statistics			
	Anoestrous	Pregnancy		Lactation Stage (wk.)			r	F-ratio	P value	Sign.
		Non-pregnant (54th d), Post-mating	Pregnant (54th d)	First	Second	Third				
	n=6	n=6	n=7							
Na	153.82±1.28	158.47±1.61	154.21±1.0	155.56±0.72	156.40±1.23	156.37±1.26	0.208 (-)	1.72	0.197	NS
K	5.47±0.14	5.74±0.15	5.69±0.19	5.62±0.21	5.56±0.19	5.43±0.23	0.224 (-)	2.02	0.164	NS
Ca	2.36±0.06	2.40±0.05	2.35±0.05	2.21±0.06	2.20±0.15	2.32±0.06	0.069 (-)	0.18	0.672	NS
P	2.45±0.08 ^{ab}	2.75±0.18 ^{c}	2.64±0.14 ^{bc}	2.62±0.13 ^{bc}	2.53±0.07 ^{b}	2.31±0.20 ^{a}	0.335 (-)	4.80	0.035	P<0.05
Mg	0.97±0.03	0.93±0.04	0.93±0.03	0.93±0.03	0.93±0.03	0.93±0.04	0.077	0.23	0.636	NS
Cl	115.40±1.19	120.82±1.79	118.16±1.11	117.96±0.48	120.34±1.07	120.86±1.14	0.200 (-)	1.59	0.215	NS
Fe	24.8±10.70	23.8 ±2.03	17.6 ±1.68	17.98±3.34	15.27±2.24	15.10±2.70	0.225 (-)	1.81	0.187	NS

** **Minerals:** The concentrations were given as mmol/ml except for Fe given as μmol/ml, NS: not significant, (-): Negative correlation, First (wk.): 5th d, second: 12th d, third: 19th d of lactation, $^{a-c}$ Means (±SEM) having different superscripts within the same row differ significantly from each other (P<0.05)*

***Table 3.** Effects of subgroups of suckling kittens (in small or large numbers) upon the serum mineral status of pregnant and lactating Van cats during breeding season*
***Tablo 3.** Emzirilen yavru alt-gruplarının (az veya çok sayılardaki) üreme sezonundaki gebe ve laktasyondaki Van kedilerinin serum mineral düzeyi üzerine etkileri*

*Minerals	Suckling Kitten Subgroups								Statistics			
	Small (n=3) (≤ 4 kittens)				Large (n=4) (>4 kittens)				r	F-ratio	P value	Sign.
	Pregnancy (54^{th} d)	Lactation (wk.)			Pregnancy (54^{th} d)	Lactation (wk.)						
		First	Second	Third		First	Second	Third				
Na	153.08±1.14	155.90±0.96	156.65±1.95	156.72±1.65	155.73±1.56	155.10±1.25	156.07±1.69	155.90±2.35	0.162	0.70	0.410	NS
K	5.88±0.25 ^{c}	5.96±0.17 ^{c}	5.65±0.29 ^{bc}	5.73±0.34 ^{c}	5.44±0.28 ^{b}	5.17±0.28 ^{a}	5.45±0.25 ^{b}	5.03±0.12 ^{a}	0.539 (-)	10.64	0.003	P<0.01
Ca	2.35±0.05	2.20±0.11	2.34±0.04	2.38±0.09	2.35±0.11	2.22±0.07	2.01±0.35	2.26±0.03	0.222 (-)	1.34	0.257	NS
P	2.67±0.21 ^{cd}	2.83±0.14 ^{d}	2.52±0.09 ^{bc}	2.58±0.28 ^{c}	2.59±0.22 ^{c}	2.35±0.10 ^{b}	2.54±0.15 ^{c}	1.96±0.11 ^{a}	0.497 (-)	8.53	0.007	P<0.01
Mg	0.93±0.01	0.94±0.05	0.89±0.02	0.94±0.04	0.94±0.07	0.91±0.05	0.98±0.07	0.92±0.09	0.038	0.04	0.849	NS
Cl	116.28±1.22	117.99±0.44	120.63±1.15	121.60±0.41	120.67±0.46	117.93±1.12	119.97±2.30	119.87±2.81	0.286	2.32	0.140	NS
Fe	18.15±2.36	21.27±5.32	12.58±1.04	16.85±4.60	16.93±2.89	13.58±2.18	18.87±4.66	12.77±1.94	0.201 (-)	1.09	0.306	NS

** **Minerals:** The concentrations were given as mmol/ml, except for Fe given as µmol/ml, NS: not significant, (-): Negative correlation, First (wk.): 5^{th} d, second: 12^{th} d, third: 19^{th} d of lactation, $^{a-d}$ Means (±SEM) having different superscripts within the same row differ significantly from each other (P<0.05)*

***Table 4.** Effects of pregnancy and lactation stage upon the serum metabolite status of Van cats during breeding season*
***Tablo 4.** Gebelik ve laktasyon sürecinin üreme sezonundaki Van kedilerinin serum metabolit düzeyi üzerine etkileri*

Metabolites	Physiological Factors						Statistics			
	Anoestrous n=6	Pregnancy		Lactation stage (wk.)			r	F-ratio	P value	Significance
		Non-pregnant (54^{th} d post-mating) n=6	Pregnant (54^{th} d) n=7	First	Second	Third				
TG, mmol/l	1.10±0.13 ^{b}	1.47±0.22 ^{c}	1.40±0.12 ^{c}	0.88±0.11 ^{a}	0.81±0.09 ^{a}	0.75±0.07 ^{a}	0.465 (-)	10.46	0.003	P<0.01
COL, mmol/l	3.27±0.41 ^{ab}	3.49±0.45 ^{b}	4.42±0.27 ^{c}	3.47±0.30 ^{b}	2.84±0.28 ^{a}	2.95±0.41 ^{a}	0.319 (-)	4.31	0.045	P<0.05
T. Prot., g/l	95.77±4.66	103.57±6.30	97.39±4.20	85.99±2.90	85.77±2.80	86.61±3.77	0.292 (-)	3.54	0.067	NS (tendency)
ALP, U/l	36.67±2.49	46.50±3.39	65.29±8.31	46.71±5.58	62.57±8.32	73.86±9.84	0.001 (-)	000	0.993	NS

***NS:** not significant, **(-):** Negative correlation, **First (wk.):** 5^{th} d, second: 12^{th} d, third: 19^{th} d of lactation, $^{a-c}$ Means (±SEM) having different superscripts within the same row differ significantly from each other (P<0.05)*

***Table 5.** Effects of subgroups of suckling kittens (in small or large numbers) upon the serum metabolite status of pregnant and lactating Van cats during breeding season*
***Tablo 5.** Emzirilen yavru alt-gruplarının (az veya çok sayılardaki) üreme sezonundaki gebe ve laktasyondaki Van kedilerinin serum metabolit düzeyi üzerine etkileri*

Metabolites	Suckling Kitten Subgroups								Statistics			
	Small (n=3) (≤ 4 kittens)				Large (n=4) (>4 kittens)				r	F-ratio	P value	Sign.
	Pregnancy (54^{th} d)	Lactation (wk.)			Pregnancy (54^{th} d)	Lactation (wk.)						
		First	Second	Third		First	Second	Third				
TG, mmol/l	1.46±0.15 ^{c}	0.94±0.15 ^{b}	0.84±0.16 ^{b}	0.84±0.10 ^{b}	1.31±0.21 ^{c}	0.82±0.18 ^{b}	0.77±0.05 ^{ab}	0.64±0.06 ^{a}	0.478 (-)	7.69	0.010	P≤0.01
COL, mmol/l	4.91±0.18 ^{d}	3.70±0.49 ^{c}	3.11±0.43 ^{b}	3.46±0.56 ^{bc}	3.76±0.22 ^{c}	3.18±0.29 ^{b}	2.48±0.28 ^{a}	2.27±0.39 ^{a}	0.651 (-)	19.08	0.000	P<0.001
T.Prot., g/l	97.87±5.67 ^{c}	90.04±3.36 ^{b}	84.78±4.61 ^{ab}	90.67±3.59 ^{b}	96.73±7.67 ^{c}	80.58±3.28 ^{a}	87.10±3.31 ^{b}	81.20±6.93 ^{a}	0.378 (-)	4.32	0.048	P<0.05
ALP, U/l	81.88±4.51 ^{cd}	55.87±6.25 ^{b}	72.50±12.90 ^{c}	88.00±12.70 ^{d}	43.17±3.81 ^{ab}	34.50±3.01 ^{a}	49.33±0.88 ^{b}	55.00±6.51 ^{b}	0.460 (-)	6.97	0.014	P≤0.01

***NS:** not significant, **(-):** Negative correlation, **First (wk.):** 5^{th} d, second: 12^{th} d, third: 19^{th} d of lactation, $^{a-d}$ Means (±SEM) having different superscripts within the same row differ significantly from each other (P<0.05)* and in lactation. Also, COL levels in pregnant queens were significantly (P<0.05) higher than those in all other occasions and further that there were significant decreases not only in the first week and but also in the second week of lactation. *(Table 4)*. Considering the number of suckling kittens, significant decreases in TG (P≤0.01) and especially in COL levels (P<0.001) were found chiefly in queens with higher number of suckling kittens *(Table 5)*.

Relationships of Minerals and Metabolites

The inter-relationships (correlations) between different minerals (*Tables 6* & *7*) and metabolites (*Tables 8* & *9*) in queens differing either in the presence of pregnancy and lactation weeks (*Tables 6* & *8*, resp.) or in the numbers of suckling kittens (*Tables 7* & *9*, resp.) are given herein. Briefly, there were numerous significant correlations for both minerals and metabolites each (varied from P≤0.05 to P≤0.001).

Correlations Between Minerals

For the relationships of minerals in queens differing in the presence of pregnancy and lactation weeks *(Table 6)*, there were significant correlations not only between P *vs.* Na (P≤0.01) and between P *vs.* K (P<0.001) but also between P *vs.* Mg (P≤0.05) and between P *vs.* Fe (P<0.01). Considering the minerals of queens differing in the number of suckling kittens *(Table 7)*, there were also significant correlations between P *vs.* both Na (P≤0.05) and K (P≤0.001) as well as between P *vs.* Fe (P<0.001).

Correlations Between Metabolites

For the relationships of metabolites of queens differing in the presence of pregnancy and lactation weeks *(Table 8)*, there were significant correlations not only between TG *vs.* COL (P<0.001) and between TG *vs.* T. Prot. (P<0.001) but also between COL *vs.* T.Prot. (P<0.01) and between COL *vs.* ALP (P≤0.05). Considering the metabolites of queens differing in the number of suckling kittens *(Table 9)*, there were also significant correlations between TG *vs.* COL (P<0.001) and between TG *vs.* T. Prot. (P≤0.01) as well as between COL *vs.* T. Prot. (P<0.001).

Table 6. *Correlations (r) between the serum mineral levels of Van cats differing in the presence of pregnancy and lactation stages*
Tablo 6. *Gebelik durumu ve laktasyon süreci yönünden farklılık gösteren Van kedilerinde serum mineral düzeyleri arasındaki ilişkiler (r)*

Minerals	Corr. (r) Groups- Pregnancy *vs.* Lactation	Minerals											
		Na		K		Ca		P		Mg		Cl	
		R	P	r	p	R	P	r	P	r	P	r	P
Na	0.208 (-)												
K	0.224 (-)	0.362	0.022*										
Ca	0.069 (-)	0.362	0.022*	0.352	0.026*								
P	0.335 (-)	0.379	0.016**	0.588	0.000***	0.164	0.312						
Mg	0.077	0.419	0.007**	0.087	0.595	0.408	0.009**	0.303	0.057*				
Cl	0.200 (-)	0.352	0.026*	0.156 (-)	0.337	0.133 (-)	0.415	0.063 (-)	0.698	0.101 (-)	0.534		
Fe	0.225 (-)	0.099	0.564	0.055	0.750	0.015 (-)	0.930	0.462	0.005**	0.322	0.055*	0.014	0.937

r= correlation, P= probability, *P≤0.05, **P≤0.01, ***P<0.001, (-): Negative correlation

Table 7. *Correlations (r) between the serum mineral levels of pregnant and lactating Van cats differing in the number (small or large) of suckling kittens*
Tablo 7. *Emzirilen yavru sayısı (az veya çok) yönünden farklılık gösteren gebe ve laktasyondaki Van kedilerinde serum mineral düzeyleri arasındaki ilişkiler (r)*

Minerals	Corr. (r) Subgroups- Small *vs.* Large Kitten Number	Minerals											
		Na		K		Ca		P		Mg		Cl	
		R	p	r	P	r	P	r	P	r	P	r	P
Na	0.162												
K	0.539 (-)	0.250	0.199										
Ca	0.222 (-)	0.334	0.083 (tendency)	0.353	0.065 (tendency)								
P	0.497 (-)	0.373	0.051*	0.606	0.001***	0.068	0.731						
Mg	0.038	0.519	0.005**	0.070	0.722	0.374	0.050*	0.218	0.265				
Cl	0.286	0.382	0.045*	0.309 (-)	0.110	0.251 (-)	0.198	0.091 (-)	0.644	0.164	0.405		
Fe	0.201 (-)	0.243	0.212	0.197	0.316	0.095 (-)	0.631	0.649	0.000***	0.426	0.024*	0.005 (-)	0.978

*r= correlation, **P=** probability, *P≤0.05, **P<0.01, ***P≤0.001, **(-):** Negative correlation*

Table 8. *Correlations (r) between the serum metabolite levels of Van cats differing in the presence of pregnancy and lactation stages*
Tablo 8. *Gebelik durumu ve laktasyon süreci yönünden farklılık gösteren Van kedilerinde serum metabolit düzeyleri arasındaki ilişkiler (r)*

Metabolites	Corr. (r) Groups-Pregnancy vs. Lactation	Metabolites					
		TG		COL		T. Prot.	
		R	P	r	P	r	P
TG	0.465 (-)						
COL	0.319 (-)	0.607	0.000***				
T. Prot.	0.292 (-)	0.597	0.000***	0.474	0.002**		
ALP	0.001 (-)	0.105	0.517	0.307	0.054*	0.010	0.949

r*= correlation,* ***P****= probability, *P≤0.05, **P<0.01, ***P<0.001,* ***(-):*** *Negative correlation*

Table 9. *Correlations (r) between the serum metabolite levels of pregnant and lactating Van cats differing in the number (small or large) of suckling kittens*
Tablo 9. *Emzirilen yavru sayısı (az veya çok) yönünden farklılık gösteren gebe ve laktasyondaki Van kedilerinde serum metabolit düzeyleri arasındaki ilişkiler (r)*

Metabolites	Corr. (r) Subgroups- Small vs. Large Kitten Number	Metabolites					
		TG		COL		T. Prot.	
		r	P	r	P	r	P
TG	0.478 (-)						
COL	0.651 (-)	0.768	0.000***				
T.Prot.	0.378 (-)	0.477	0.010**	0.679	0.000***		
ALP	0.460 (-)	0.290	0.135	0.349	0.069 (tendency)	0.221	0.258

r*= correlation,* ***P****= probability, **P≤0.01, ***P<0.001,* ***(-):*** *Negative correlation*

DISCUSSION

Mineral deficiencies are reported to cause numerous disorders, including the low milk yield and reduced fertility in lactating animals [14]. Prepartum normal development of foetus and its subsequent growth and postpartum health of newborn are essential for a given individual. Hence, it is a pre-requisite for lactating/suckling mother to consume all nutrients and minerals in a balanced fashion to feed its offsprings [27] sufficiently. Therefore, in the last trimester (one-third) of pregnancy, the diet should contain high amounts of Cu as well as other trace elements [28].

Serum mineral concentrations (except Mg) of non-pregnant (post-mating) queens were higher -more or less- than those in pregnant ones, even before parturition (lactation), as this was particularly the case for P levels. Indeed, the presence of pregnancy affects numerous metabolisms including those of macro minerals. Also, trace elements, particularly those needed during pregnancy, take roles in the metabolism and storage of proteins. Some minerals bind to the conventional transport proteins to the specific metals and ensure their transfer to target sides in the organism [29,30].

Na levels remained almost stable. However, the levels were numerically higher in non-pregnant individuals (no need to support foetal development or no excretion by milk yield). Additionally, Na could easily be obtained from all the salts and is a basic element for the body. Kidneys excrete the excess amount, but it is retained by renal filtration in deficit. Apparently, the commercial cat diets contain sufficient amounts of Na (0.06% per 100 g of diet herein), and hence its deficiency would be seen rarely.

K is a cation, playing various roles in the intracellular fluid, acid-base balance, nervous impulses, muscular functions, especially the cardiac muscle and Na^+/K^+-ATPase functions. It is also needed for glycogenesis. In hypokalaemia, marked reduction in milk yield, metabolic alkalosis, paralysis and diarrhoea could occur in dairy cows [31,32]. However, unlike with Na, there were marked ($P<0.05$) fluctuations in its levels, as the number of suckling kittens increased (up to 7 kittens) observed herein. This further led to more and more excretion of P that was markedly ($P\leq0.001$) correlated with K. It was also tended ($P=0.065$) to correlate with Ca that has being gradually excreted during suckling. Although this may indicate hypokalaemia (presumably leading to alkalosis) that might have occurred in suckling queens, as with dairy cows, due to higher number of kittens. But, it was compensated by metabolic adaptations, as presumably facilitated by higher amount of feed intake containing K.

Additionally, there was a constant but only steady decrease in the level of Ca, as normally having a close connection with P [4]. The concentrations of plasma Ca (particularly the ionised form) and P are regulated not only by parathyroid hormone (PTH), cholecalcipherol (vitamin D_3) and calcitonin hormones (tyrocalcitonin: CT), but also by dietary factors. Other hormones such as oestrogens, corticosteroids, growth hormone, glucagon and thyroxine may also influence the calcium homeostasis. Indeed, oestrogen and growth hormone hasten the Ca^{2+} absorption [33,34]. The need for Ca intake increases during the pregnancy and lactation. Undoubtedly, Ca reserves deplete rapidly as the embryo develops during the pregnancy and eventual lactation leads to increasing amounts of its output [33-35]. Although dairy cows with high milk yield would be more prone to Ca deficiency, this was not essentially the case herein.

P is involved in the structural/functional events of ATP, nucleic acids, bones and teeth [36]. It is known that Ca concentration is closely inter-linked with P. Vitamin D is effective on P absorbtion [37]. In our study, there was marked ($P<0.05$) reduction in P value as the lactation period prolonged up to 3 weeks. Indeed, its levels either markedly decreased in queens with small number of suckling kittens or fluctuated in those with large number of kittens. It was further observed that the P concentrations in non-pregnant queens tended initially to be lower after parturition (early lactation).

Although the increasing amounts of milk yield inevitably lead to reduced elemental levels (especially P herein), but they could be replaced by some degree of mobilisation from the bones. Indeed, in the present study, Ca levels slightly increased by the 3rd week of lactation following the earlier numerical decreases. It is considered that, P concentration would also probably rise again subsequently, through endogenous stores, to re-gain homeostasis needed for the later stages of lactation.

Mg is required for ATP formation, transportation of Na and K through the cellular membranes [38]. Its dietary level is influenced by some factors, such as protein content of diet and body Mg status/stores of a given animal. High Ca/P intake decreases its absorption [4]. Since Mg concentration was affected neither by pregnancy nor by lactation herein (remaining the most stable mineral studied), its deficiency could well be avoided further by dietary protein and Mg when given in a balanced fashion, even in those queens with high number of suckling kittens.

Cl levels of queens with small number of kittens became numerically higher as the lactation period proceeded, while there was only a slight decrease by the first week of lactation, followed by steady increase in queens suckling large number of kittens. It was considered that, this was mainly because of re-gaining the physiological levels earlier in queens with small litter size.

Considering the changes in Fe levels, there were consistent but only numerical decreases starting from the presence of pregnancy and continuing as the lactation period proceeded. Further, the demand for Fe increases especially during the pregnancy and lactation for large kitten size. It is known that Fe has vital metabolic functions in blood formation and oxygen transfer. Apparently, in pregnant and lactating queens especially those carrying/suckling large kitten sizes would hasten the depletion stage of Fe stores, leading to a greater risk of anaemia to occur. Indeed, it was reported that normocytic normochromic anaemia is frequently observed especially in the last one-third of pregnancy in queens [35]. Hence, we may recommend for cat breeders greater to provide amount of Fe-containing diets or supplements for pregnant queens and especially for those suckling greater number of kittens.

In the literature, mineral studies in non-pregnant and pregnant rabbits showed that there were no marked differences for Na, K, Ca, P and Cl levels [39], as generally in parallel with the present results. However, herein, given the consistent numerical reductions for the majority of minerals studied (Na, K, Ca and Cl), P and Fe levels (with marked interactions together) were relatively (but not statistically) lower in pregnant queens while Mg levels remained unchanged, as compared to those in non-pregnant queens. Furthermore, the marked interactions between P and Fe levels in earlier stages of lactation were more apparent as the suckling by greater number of kittens was prolonged by later weeks. Differences of minerals between the two species might be related to differences in the stages and/or durations of pregnancy, feeding characteristics (plant- or meat-based) and contents of diet used, etc.

In fact, all the minerals within the metabolism are inter-linked with each other. Thus, they are expected to affect each other's absorption and excretion. Indeed, this is especially so for Na and K, Ca and P as well as Mg and Cl. According to our findings, the minerals had marked (ranging from $P\leq0.05$ to $P\leq0.001$) inter-relationships with each other during pregnancy, early lactation weeks and suckling by different number of kittens in queens. Herein, it was observed that there were marked correlations between P *vs.* Na ($P\leq0.05$ - 0.01) and between P *vs.* K ($P\leq0.001$ all) in all occasions (*i.e.* pregnancy, lactation and suckling) and also between Cl *vs.* Na ($P<0.05$) in different number of suckling kittens. It was considered that, given the requirements of metabolism, such an interaction observed between the minerals could be regarded as physiologically normal to sustain homeostasis in any way.

Regarding the metabolites studied, it was observed that TG levels in non-pregnant/pregnant queens were markedly ($P<0.05$) greater than those both in anoestrus and in lactation. Likewise, COL levels in pregnant queens were markedly ($P<0.05$) greater than those in all other occasions given and further that there were marked reductions not only in the first week and but also in the second week of lactation. Further, TG and especially COL levels markedly reduced in queens with higher number of suckling kittens.

The initial marked ($P<0.05$) rise in TG concentrations was then followed by marked ($P<0.05$) reductions during anoestrus, pregnancy and lactation. Likewise, COL levels, following the initial marked rise in pregnancy also markedly ($P<0.05$) decreased in lactating queens. Undoubtedly, the both parameters are crucial for pregnancy such that they have critical functions both in meeting the energy requirement and sustaining the hormonal homeostasis. Of them, COL is needed for the synthesis of progesterone that, with its steroid structure, is responsible mainly for sustainable embryogenesis/pregnancy [20]. Herein, although its concentration was higher during pregnancy, but these high levels dropped dramatically following parturition, namely by the 5th d of lactation, as expected. In the literature, Haneda et al.[39] observed that the amount of COL decreased gradually in rabbits, as the period of pregnancy prolonged. In our study, although the amounts of TG and COL were higher during pregnancy, as compared to those in anoestrous and non-pregnant (post-mating) queens, their levels reduced as the lactation proceeded. Differences in the levels of these metabolites between queens and rabbits might be related to rigid differences in the sexual cycles and pregnancy periods of the species concerned.

T. Prot. levels in lactating queens with higher number of suckling kittens negatively markedly ($P<0.05$) fluctuated, while ALP *vice versa* ($P<0.05$). However, the levels both only numerically reduced, regardless of the number of suckling kittens. However, there were marked relationships between COL *vs.* TG ($P<0.001$), between COL *vs.* T.Prot. ($P<0.01$) and between COL *vs.* ALP ($P\leq0.05$) as well as between T.Prot. *vs.* TG ($P<0.001$) during pregnancy and lactation periods. Additionally, when all the metabolites (of TG, COL, T.Prot. and ALP) studied were categorised according to the number of suckling kittens, marked differences ($P\leq0.01$, $P<0.001$, $P<0.05$ and $P\leq0.01$, resp.) were observed between the two suckling kitten groups. In the literature, Haneda et al.[39] reported in rabbits that although ALP levels remained unchanged in non-pregnant females, but it dramatically reduced as the period of pregnancy prolonged. These results are in parallel with the present findings. Also, ALP levels relatively consistently increased further during later stages of lactation. It was known that higher enzyme activity indicate not only the ultimate outcome of increased hepatic function but also higher progesterone levels available in rabbits [40]. In fact, the interactions between the metabolites, like minerals, are physiologically normal, just as their potent structures to influence each other's syntheses and functions, either in synergetic, supportive or inhibitory way.

Animals, like those housed in the Van Cat Centre, consume their daily allowances generally including the essential nutrients in a balanced fashion [41,42]. However, dietary supplements could be beneficial, particularly for those animals during breeding, pregnancy and lactation periods, since they spend extra energy during these physiological states. Undoubtedly, mineral deficiency may occur more commonly due to the increased milk production and higher dry matter intake during lactation period [42,43]. Instead of the supplementation of dietary nutrients such as by vitamins and minerals separately, it is recommended that they should be given in combination to benefit more from their synergetic effects [24,42,44]. Furthermore, in cats and dogs, it has been reported recently that with the consumption of home-made diet mixture, the likelihood of mineral substance deficiencies could be quite rare during the periods of breeding, pregnancy and lactation [42], thus feeding queens should receive more concern to avoid any further risk of mineral or metabolite deficiencies especially when they are fed with standard cat diet only, as was the case herein.

In conclusion, the present findings suggest that; i) unlike other minerals (Na, K, Ca, Mg, Cl and Fe) remaining virtually unchanged, there were marked alterations in the concentrations of K (negative fluctuation) and P (reduction) and ii) all the metabolites studied (TG, CHOL, T.Prot. and ALP) also markedly changed, particularly when the lactation period proceeded and the number of suckling kittens increased in Van cats during the normal breeding season. Undoubtedly, the likelihood of mineral and metabolite deficiencies and their proportional imbalances would increase eventually in lactating queens particularly with prolonged lactation and greater numbers of suckling kittens. Therefore, it was recommended that; i) the requirements of trace elements/minerals and metabolites, that would inevitably increase physiologically during the pregnancy and lactation, should be balanced by giving proper (enriched quality and/or higher quantity) commercial diet in daily allowance, as needed and ii) since the requirements of essential macroelements (P and K) and main metabolites (TG, CHOL, T.Prot. and ALP) increase not only in the last trimester (one-third) of pregnancy, but also the during prolonged lactation, diets should also be supplemented by these elements and metabolites during the pregnancy and lactation in Van cats in breeding season.

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