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Calcium and phosphorus metabolism in peripartal dogs

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Abstract

Recommended allowances for calcium and phosphorus are mostly based on factorial calculations partly set at the level determined adequate for giant breeds (Nutrient requirements of dogs and cats. Washington, DC, USA: The National Academies Press. 2006). Information about appropriateness of supply with both minerals during the peripartal phase is limited. From other species is known that bone mineral stores are used in addition to oral intake of calcium and phosphorus in periods of higher needs such as gestation and lactation. The aim of this study was to determine parameters of calcium and phosphorus homeostasis in female dogs receiving the recommended amount of these minerals according to NRC (Nutrient requirements of dogs and cats. Washington, DC, USA: The National Academies Press. 2006) during the peripartal phase. In five Beagles and four Foxhound crossbreds, all primiparous with a litter size of 1–8 puppies, apparent digestibility of calcium and phosphorus as well as serum parameters of mineral metabolism (total and ionised calcium, phosphorus, parathyroid hormone, bone specific alkaline phosphatase, crosslaps) was determined in the period of 12–9 days before and 4–9 days after parturition. The apparent digestibility of calcium was relatively low and did not differ significantly between both peripartal phases, whereas the apparent digestibility of phosphorus increased during lactation. Serum concentrations of calcium (total as well as ionised), phosphorus and parathyroid hormone did not differ between gestation and lactation. The bone resorption marker serum crosslaps increased in lactating dogs but most individual values were within the reference range for adult female dogs at maintenance. On the other hand, the bone formation marker bone specific alkaline phosphatase decreased from prepartal to postpartal phase with values clearly above reference range in both phases. Based on the results especially of the bone markers, which stayed within the reference range during the peripartal phase without indicating predominant bone resorption, we hypothesise that the applied recommended daily allowances defined for peripartal dogs are appropriate.

KEYWORDS
calcium, gestation, homeostasis, lactation, phosphorus

1 | INTRODUCTION

It is a well-known fact that during lactation and gestation various species use their bone mineral stores in addition to oral supply with calcium and phosphorus in order to meet the increased demand. This applies for example to dairy cows, where the calcium and phosphorus homeostasis during reproduction was intensively studied (Beighle, 1999; Holtenius & Ekelund, 2005; Horst, Goff, & Reinhardt, 2005; Rowland, Capen, Young, & Black, 1972). It was demonstrated that also in cats bone resorption occurs in the peripartal phase (Liesegang & Wichert, 2012). In Beagles, it was shown that increased intracortical bone remodelling occurs during lactation (Miller, Omura, & Miller, 1989; Vajda, Kneissel, Muggenburg, & Miller, 1999). However, there is a lack of studies about the correlation between calcium and phosphorus intake and digestibility of both elements in the peripartal period on one hand and serum parameters of mineral metabolism and bone turnover on the other.

A reduction of postpartum blood calcium is physiological in several species (e.g. Kincaid, 2008 [dairy cows], Liesegang & Wichert, 2012 [cats], Mariella, Pirrone, Gentilini, & Castagnetti, 2014 [horse], Wilkens et al., 2014 [goat, sheep, dairy cows]) but may have a higher prevalence than anticipated because the animals might not show clinical signs (Reinhardt, Lippolis, McCluskey, Goff, & Horst, 2011). In some species, an absolute calcium deficiency might cause decreasing calcium blood levels, that is subclinical and clinical hypocalcaemia respectively. In other species, it is the sudden demand for calcium at the onset of lactation, which causes this situation. In reproducing females, this is known as eclampsia or periparturient paresis respectively. The pathophysiological pathway differs between the species. An absolute calcium deficiency with a calcium intake below requirements might cause similar clinical signs as an insufficient adaptation of the calcium metabolism when lactation sets in. The periparturient hypocalcaemia of dairy cows, usually referred to as milk fever and defined as an adaptive problem, is a good example for the latter situation (Goff et al., 1991; Horst et al., 2005; Rowland et al., 1972). Here, both renal production of 1,25-dihydroxy vitamin D3 (1,25(OH)$_2$D3) and the osteoclasts seem to be refractory to PTH stimulation (Goff et al., 1991). Rats also do not show increased bone turnover until lactation (Halloran & DeLuca, 1980). In sheep, depending on the breed, a prepartal hypocalcaemia was shown, but dairy sheep can also develop a disease comparable to milk fever (Oetzel, 1988). In humans, an increased bone turnover can be seen both during gestation and lactation (Sowers et al., 1993; Yamaga, Taga, Minaguchi, & Sato, 1996), which also cannot be amended by higher oral calcium supply (Cross, Hillman, Allen, Krause, & Vieira, 1995). In cats, an increased bone resorption during late gestation and early lactation was demonstrated by measuring osteoclastic bone marker serum crosslaps and measuring the bone mineral density using the DEXA method (Liesegang & Wichert, 2012).

In dogs, reports of eclampsia or puerperal tetany phase exist but are less frequent than in other species (Pathan et al., 2011). Reports show that small or toy breeds are overrepresented (Pathan et al., 2011) as well as dogs with large litters (Drobatz & Casey, 2000). A loss of stabilising membrane bound calcium leads to an increased permeability of the membranes, which are therefore more easily depolarised. This may then lead to restlessness, nervousness or neuromuscular tetany (Pathan et al., 2011). A sufficient supply with calcium and phosphorus aiming at prevention of this condition and excessive bone resorption is warranted. Even though the role of 1,25(OH)$_2$D3 and PTH in dogs with eclampsia is not entirely defined, some authors recommend additional supplementation with vitamin D (Pathan et al., 2011). Existing recommendations for calcium and phosphorus supply in reproducing female dogs are largely based on factorial calculations (DLG, 1989; Meyer, 1984; Meyer, Dammers, & Kienzle, 1985; NRC, 2006). Compared to maintenance, they are higher during the peripartal period to cover for increased requirements for tissue accretion in gestation (foetal growth, especially skeletal development) and milk production during lactation. Until 35 days ante partum the overall nutrient requirements for dogs during gestation do not differ from maintenance (Meyer et al., 1985; NRC, 2006). In the following phase of late gestation, the recommendations for energy, protein as well as mineral and vitamin supply are increased (DLG, 1989; Meyer et al., 1985; NRC, 2006). During lactation, additional energy, protein, calcium and phosphorus for milk production are needed. The milk yield is positively correlated in a curvilinear manner to the number of suckling puppies. Consequently, the requirements during lactation are depending on the litter size when calculated using a factorial approach (Meyer et al., 1985).

A meta-analysis about short-term studies (1–6 weeks) on apparent digestibility of calcium in dogs has shown that adult dogs at maintenance do not adapt their intestinal calcium absorption to different amounts of calcium in their diets (Mack, Alexander, Morris, Dobenecker, & Kienzle, 2015). Even dogs fed a low calcium diet for 6 months failed to decrease their faecal calcium losses (Schmitt et al., 2017). In times of adequate calcium intake renal calcium losses are low (10–15 mg/kg bodyweight [BW]) and are further reduced only slightly in case of low calcium intake (to ~5 mg/kg BW) (Chen & Neuman, 1955; DLG, 1989). Thus, decreasing the calcium losses via urine is a negligible way for dogs to maintain calcium homoeostasis. To maintain their calcium homoeostasis in case of an inadequate intake, adult dogs at maintenance therefore have to use their skeletal stores to a higher degree (Böswald, Dobenecker, Clauss, & Kienzle, 2018; Schmitt et al., 2017). In contrast, the phosphorus homoeostasis is regulated to a higher extend by renal excretion and use of tissue stores (Kiefer-Hecker, Kienzle, & Dobenecker, 2018).

The aim of this study was to investigate parameters of calcium and phosphorus metabolism during the peripartal phase in dogs to test if either the recommended allowances for both major minerals are high enough to prevent effects on the parameters such as increased bone resorption or the dogs are using their skeletal stores to meet the increased demand during gestation. Our underlying hypothesis was that dogs in the peripartal phase are not able to efficiently increase the apparent digestibility of calcium and phosphorus. Together this would allow to comment on the adequacy of the current recommendations.

2 | ANIMALS, MATERIALS AND METHODS

Five female Beagles (14 ± 1.9 kg BW) and four female Foxhound Boxer Ingelheim Labrador crossbreds (FBI; 33 ± 6 kg BW) were available for this study. Protection against parasites, parvovirosis, distemper, canine infectious hepatitis, kennel cough and leptospirosis was provided. Starting on the 5th day of the heat serum progesterone, concentration was measured every other day to determine the time of ovulation. On the day of ovulation and the following three days, the male and the bitch mated 2–3 times per day. Three dogs were inseminated artificially two days after estimated ovulation. The approximate date of birth was calculated for the day 63 after ovulation.

In the last 3 weeks of gestation, all dogs were housed individually during the night in kennels of 9–19 m^2 with visual contact to other dogs. At daytime, they had access to large outdoor runs (29–100 m^2) in their usual groups of 3–7 dogs for 4–8 hr, depending on weather conditions, their individual activity level and welfare aspects during late gestation. During the balancing trial in late gestation and the whole lactation period, the dogs were housed individually and with their litter, respectively, and were taken for walks individually. The start of the first balance trial was planned for day 12–9 ante partum because from this time point onwards the bitches were housed individually to provide a calm place separated from the other dogs of the group before giving birth. This separation enabled a quantitative sampling of faeces in every individual. The second trial started 4 days postpartum assuming the bitches manage a food intake normal for early lactation. A later balancing trial during peak lactation (week 3–5; Rüsse, 1961) would have required a separation of bitch and its puppies. This was avoided for welfare reasons and to prevent impact by lower milk intake of the puppies. Both trials lasted for five consecutive days.

To ensure a sufficient energy and nutrient intake, during late gestation and early lactation different complete and balanced commercial feedstuffs either for reproduction or for all stages were offered ad libitum in different bowls. The bitches could choose according to their preferences while aiming to meet or exceed all nutrient requirements. Two to three times a day the bowls were inspected and refilled if necessary, leftovers were weighed back after 24 hr to calculate daily intake of energy and nutrients. The intake was then compared with the recommended allowances defined for gestating and lactating bitches of the respective BW (NRC, 2006). For gestation and lactation, the energy requirements were calculated with the equation ME (kcal) = 130 kcal × kg $BW^{0.75}$ + 26 kcal × kg BW (NRC, 2006) for gestation and ME (kcal) = 145 kcal × $BW^{0.75}$ + BW × (24n + 12m) × L (NRC, 2006) for lactation (n = number of puppies 1–4; m = number of puppies 5–8; L = correction factor for stage of lactation). To ensure that calcium, phosphorus and further nutrient requirements were met or exceeded, the intake was compared with the recommendations of the NRC (2006). The recommended daily allowances for calcium and phosphorus were calculated for every individual based on the average energy requirements for every bitch using her actual BW and the recommended amounts of minerals in reproducing dogs per unit of metabolisable energy (1,900 mg calcium/1,000 kcal; 1,200 mg phosphorus/1,000 kcal).

A sample of each food was freeze-dried and ground for later analyses. Faeces were collected quantitatively, weighed, freeze-dried, mixed and ground. Calcium levels in food and faeces were analysed by flame-emission photometry (Eppendorf Flammenphotometer EFOX 5,053, HJG Spezialmesssysteme), phosphorus levels by spectrophotometry (GENESYS 10 UV, Thermo Spectronic), both after acid hydrolysis and wet digestion in a microwave (Janssen, Matter, Rieß, & Seifert, 2006).

At one day during each balancing trial, a blood sample was taken in the overnight fasted animal at around 8 a.m. from the V. cephalica antebrachii. Serum samples were used to measure PTH, ionised calcium, total calcium, phosphorus, bone alkaline phosphatase (bALP) and serum crosslaps. For PTH measurement, 0.5 ml of the serum was transferred into transport tubes with a PTH stabiliser composed of a mixture of several proprietary protease inhibitors (ALOMED). For detection of ionised calcium, total calcium and phosphorus, 0.7 ml serum was transferred into transport tubes of the same external laboratory (ALOMED) ensuring low oxygen contact. Aliquots of the remaining serum were transferred to safe-lock tubes (Eppendorf) and stored at −80°C for determination of bALP and serum crosslaps.

The serum samples for determination of PTH, ionised calcium, total calcium and phosphorus were shipped to a diagnostic laboratory specified on veterinary diagnostics (ALOMED). Total calcium was analysed by colorimetry via endpoint determination using the cresolphthalein method; phosphorus was determined using the phosphor-molybdat method (ILab 650 analytic system, IL GmbH). The reference range of the laboratory is 2.3–2.8 mM for total calcium and 1.0–1.7 mM for phosphorus in adult dogs respectively. Ionised calcium was measured using a calcium ion-selective electrode (CRT 8 Nova biomedical) with simultaneous assessment of serum pH to correct for the fixed pH value of 7.4 (standardised ionised calcium). The reference range of the laboratory for ionised calcium in dogs is 1.29–1.55 mM. For PTH determination, a direct luminometric sandwich immunoassay (ILMA) validated for dogs was used (Schmitt et al., 2017). The reference range of the laboratory for PTH is 8–45 pg/ml in adult dogs. Serum crosslaps were measured with a human ELISA (immunodiagnostic systems Serum CrossLaps® ELISA; Immunodiagnostic Systems GmbH) validated for dogs. In literature, the reference range for crosslaps measured by ELISA is 0.11–1.83 ng/ml in intact female dogs (Belić et al., 2012). BALP was determined by ELISA for human bALP from MicroVue™ Quidel® BAP Enzyme-Immunoassay (TECOmedical AG), a method validated for dogs (Allen, Allen, Breur, Hoffmann, & Richardson, 2000). The reference range for this ELISA kit in adult dogs of 3–7 years is given with 0.9 ± 0.11 U/L (Belić et al., 2010) and 6.7 ± 3.6 U/L (Allen et al., 2000). Repeated measurements were conducted for both parameters measured in-house.

All procedures and protocols were conducted in accordance with the European guidelines of the Protection of Animals Act. The study was approved by the representative of the Chair of Animal Welfare of the Faculty of Veterinary Medicine of the Ludwig-Maximilians Universität München as well as the Government of Upper Bavaria (reference number AZ 55.2-1-54-2532.3-6511).

2.1 | Statistical methods

Data are expressed as mean ± standard deviation. In normally distributed parameters, a one-way RM ANOVA was carried out to test for period gestation or lactation. A statistical difference between the groups was considered significant with $p < .05$. The software used for statistical analysis was SigmaPlot 12.5 (Systat Software GmbH).

3 | RESULTS

During gestation, the dry matter intake amounted to 682 ± 268 g/day with an average energy supply of 1.2 ± 0.3 MJ ME/kg $BW^{0.75}$, exceeding the recommended intake (NRC, 2006; 167.1 ± 43.3%) in all dogs. There was no correlation between the number of foetuses and the amount of dry matter and energy intake respectively. In this phase, the calcium and phosphorus intake amounted to 953 ± 236 mg/kg $BW^{0.75}$ and 735 ± 170 mg/kg $BW^{0.75}$, respectively (Table 1) and therefore exceeded the recommendations in all cases (NRC, 2006; calcium 271% ± 65% phosphorus 331% ± 78%).

All dogs were primiparous with 5 ± 3 puppies. In eight out of nine cases, the bitches gave birth naturally, one needed a caesarean section. During the surgery, this bitch was also spayed and received a blood transfusion. Otherwise, all bitches remained clinically healthy, as assessed by weekly veterinary health checks. All puppies grew up normally and healthy as assessed by daily weighing and daily health checks.

During the first week of lactation, the dry matter intake amounted to 717 ± 419 g/day leading to an average energy intake of 1.2 ± 0.5 MJ ME/kg $BW^{0.75}$ with an energy consumption exceeding the calculated requirements for lactation in two cases and an intake below the calculated amounts in three cases. The other four bitches showed an energy intake matching the average requirements stated by NRC (2006) rather well. The calcium and phosphorus intake amounted to 1,039 ± 407 mg/kg $BW^{0.75}$ and 863 ± 318 mg/kg $BW^{0.75}$, respectively, (Table 1) corresponding to 177 ± 52 and 331.8% ± 78.9% of the recommendations for calcium and phosphorus (NRC, 2006) respectively.

During late gestation, the apparent digestibility of calcium and phosphorus amounted to 12% ± 22% and 35% ± 15% respectively. The apparent digestibility of both elements was not influenced by litter size (Table 1). In three out of nine dogs, the daily faecal calcium losses exceeded the daily intake. This led to a negative apparent calcium digestibility and caused the high variation between the dogs. In contrast, the apparent phosphorus digestibility was positive in all dogs, demonstrating a phosphorus accretion.

The balance trial conducted during early lactation revealed a trend for increased apparent digestibility for calcium (24% ± 18%), while the apparent digestibility of phosphorus increased significantly (48% ± 10%; $p = .019$). The litter size did not have an influence

TABLE 1 Intake, faecal excretion, apparent digestibility and apparently digested calcium and phosphorus in dogs during gestation and lactation

		Intake [mg/kg $BW^{0.75}$]		Faecal excretion [mg/kg $BW^{0.75}$]		Apparent digestibility [%]		Apparently digested [mg/kg $BW^{0.75}$]*	
	n	Ca	P	Ca	P	Ca	P	Ca	P
Gestation									
Total	9	953 ± 236	735 ± 170	815 ± 193	468 ± 120[a]	12 ± 22	35 ± 15[a]	138 ± 203 (−183/374)	267 ± 140[a] (39/439)
Small litter (≤4 puppies)	5	865 ± 260	666 ± 197	744 ± 229	445 ± 127[a]	12 ± 24	31 ± 17[a]	121 ± 195 (−91/357)	221 ± 137[a] (39/352)
Large litter (>4puppies)	4	1,063 ± 171	822 ± 85	903 ± 124	497 ± 61[a]	13 ± 23	39 ± 14[a]	160 ± 242 (−183/374)	325 ± 140[a] (149/439)
Lactation									
Total	9	1,039 ± 407	863 ± 318	757 ± 310	439 ± 182[b]	24 ± 18	48 ± 10[b] P= .019	281 ± 285 (−21/862)	423 ± 198[b] (146/812) P= .019
Small litter (≤4 puppies)	5	810 ± 257	676 ± 184	599 ± 114	347 ± 58[a]	22 ± 20	47 ± 13[a]	211 ± 227 (−21/529)	329 ± 163[a] (146/542)
Large litter (>4 puppies)	4	1,324 ± 399	1,097 ± 305	956 ± 240	555 ± 169[a]	26 ± 19	49 ± 8[a]	368 ± 360 (6/862)	542 ± 187[a] (388/812)

Note: Means in one column not sharing a superscript letter are significantly different, $p < .05$.

*Apparently digested calcium and phosphorus: range given in brackets.

TABLE 2 Serum total calcium, ionised calcium, phosphorus and PTH in dogs during gestation and lactation

	n	Total Ca	Ionised Ca	P	PTH
		mM			pg/L
Reference range[a]		2.3–2.8	1.29–1.55	1.0–1.7	8.0–45.0
Gestation					
Total	9	2.4 ± 0.1	1.4 ± 0.0	1.8 ± 0.2	18.4 ± 10.3
Small litter (≤4 puppies)	5	2.4 ± 0.1	1.4 ± 0.1	1.7 ± 0.2	16.8 ± 13.0
Large litter (>4 puppies)	4	2.4 ± 0.1	1.4 ± 0.0	1.9 ± 0.2	20.0 ± 4.4
Lactation					
Total	9	2.4 ± 0.1	1.4 ± 0.0	1.7 ± 0.3	19.2 ± 8.9
Small litter (≤4 puppies)	5	2.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.2	15.8 ± 9.1
Large litter (>4 puppies)	4	2.4 ± 0.1	1.4 ± 0.1	1.7 ± 0.3	23.5 ± 7.3

[a]Laboratory reference range.

on the apparent digestibility of both minerals (Table 1). During lactation, the apparent calcium digestibility was clearly positive in all but one dog; the apparent phosphorus digestibility remained positive in all dogs. No statistically significant correlation existed between litter size and apparent calcium digestibility.

Serum concentrations of total calcium, ionised calcium and phosphorus stayed within the reference range in all dogs in both phases without differences between late gestation and early lactation and litter size respectively (Table 2). Mean serum PTH concentrations stayed within the reference range of the laboratory in both phases without significant influence of the litter size (Table 2). In one bitch, PTH concentrations were below the reference range in both phases without any clinical signs.

During late gestation, serum crosslaps, measured as marker of bone resorption, amounted to 0.6 ± 0.7 ng/ml and increased significantly to 1.3 ± 0.6 ng/ml in early lactation ($p = .008$; intra-essay coefficient of variation 9.6%; Figure 1). In seven out of eight bitches where crosslaps were determined, serum concentrations were within the reference range, only in one bitch this parameter was above the given range (2.1 ng/ml, range: 0.11–1.83 ng/ml). During lactation, a second bitch had crosslaps values increased above the given range (2.3 and 2.2 ng/ml). The mean serum concentrations of the bone formation marker bALP were above both reference ranges (0.9 ± 0.11 U/L and 6.7 ± 3.6 U/L) in both phases but decreased significantly during the first week of lactation (27.6 ± 8.5 vs. 19.5 ± 5.7 U/L; $p = .035$) (Figure 2). The intra-essay coefficient of variation for the measurement of serum crosslaps was 3.4%. There was no effect of litter size neither on crosslaps nor on bALP.

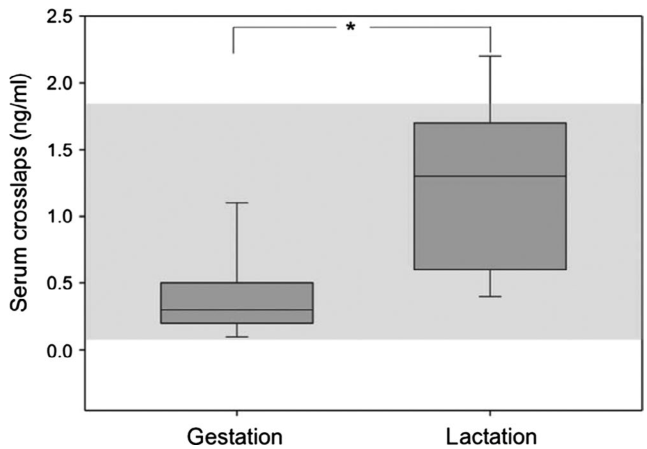

FIGURE 1 Serum crosslaps concentration [ng/ml] in dogs during gestation and lactation. Reference range 0.11–1.83 ng/L (marked as grey area); $p < .05$

4 | DISCUSSION

All bitches were fed commercial complete moist and dry diets ad libitum aiming at an energy and mineral intake to meet or exceed requirements (NRC, 2006). Accordingly, the actual energy intake during late gestation also met and in some cases exceeded the calculated requirements. The high feed intake led to a calcium and phosphorus intake above the recommended daily allowance (DLG, 1989; FEDIAF 2018; Meyer et al., 1985; NRC, 2006). Independent of the number of foetuses, the apparent digestibility of calcium and phosphorus remained relatively low in the gestating bitches when compared to values given for adult dogs during maintenance (−20% to 44% vs. 5% to 50% apparent digestibility of calcium; 8%–54% vs. 46%–90% apparent digestibility of phosphorus; DLG, 1989). Schmitt et al. (2017) also found low and even negative values for the apparent digestibility of both elements in adult dogs during maintenance (−134% to 18% apparent digestibility of calcium, −28% to 49% apparent digestibility of phosphorus). Because growth is another period of increased calcium and phosphorus demand due to skeletal development, data from puppies were compared with results from the present study in peripartal dogs. Dobenecker (2002) as well as DLG (1989) report of an apparent calcium digestibility of 40%–90% and an apparent phosphorus digestibility of 50%–90%. Therefore, the measured values in the bitches of this trial were lower than expected and in three cases even a negative calcium balance, that is a negative value for the apparently digested mineral, was observed (Table 1). The amount of apparently digested phosphorus was positive in all animals. Instead of these moderate values for apparently digested calcium and phosphorus, only one bitch with a large litter

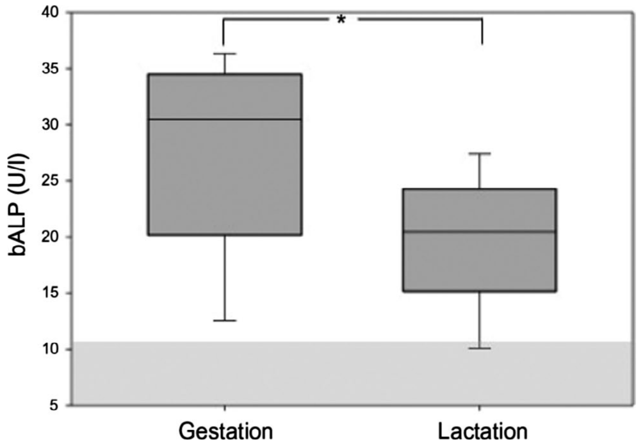

FIGURE 2 Bone alkaline phosphatase (bALP) [U/L] during gestation and lactation. Reference range 6.7 ± 3.6 U/L (marked as grey area); $p < .05$

of eight puppies showed a slightly increased concentration of the bone resorption marker (crosslaps) above the reference range during gestation. In this trial, bALP was used as one possible marker of bone formation. As the blood sampling occurred always at the same time, every dog could be used as its own control to compare both periods of the reproduction cycle. As bALP and other parameters show diurnal variances, the absolute values might have differed at different times of blood sampling (Allen, 2003). However, the values for bALP were clearly above the reference range of this parameter in all dogs of this trial during the gestation period. Maybe this was due to increased bone formation in the foetuses. These data suggest a high bone turnover including a high bone formation as expected due to foetal bone development. Determination of further markers of bone turnover, as recommended by Allen (2003), might have been useful. The individual serum concentrations of total and ionised calcium as well as phosphorus were in the reference ranges. None of the bitches showed PTH values above the reference range.

In the balance trial conducted 5–10 days after parturition, some bitches realised an energy intake below the average energy requirement given by NRC (2006). Possible reason for this below-expected food intake might be due to individual circumstances such as post-surgery condition or stress in primiparous bitches. However, in all but one bitch the intake of calcium and phosphorus exceeded the recommended intake during lactation (calcium: 601 ± 171 mg/kg $BW^{0.75}$ ≙ 177% ± 52% recommended daily allowance; phosphorus: 379 ± 108 mg/kg $BW^{0.75}$ ≙ 234% ± 69% recommended daily allowance; NRC, 2006). The apparent digestibility of both minerals increased slightly but not significantly between gestation and lactation, leading to increased amounts of apparently digested calcium and phosphorus (138 ± 203 vs. 281 ± 285 mg Ca/kg $BW^{0.75}$; 267 ± 14 vs. 423 ± 198 mg P/kg $BW^{0.75}$). The increase in apparently digested phosphorus was significant (p = .02). After whelping, the apparent digestibility of both minerals was similar to the data published by Meyer et al. (1985). Only in one bitch the value for the apparently digested calcium remained negative. The serum concentrations of crosslaps increased significantly (p = .008) while the values exceeded the reference range only in two individuals. This increased bone resorption during early lactation was also found in cats (Liesegang & Wichert, 2012) and could not be prevented by a realised intake of both minerals in excess to the recommended daily allowances given by NRC (2006) and a positive mineral balance in eight of the nine bitches. This was also shown in other species (cow: Beighle, 1999, Holtenius & Ekelund, 2005, Horst et al., 2005, Rowland et al., 1972; man: Sowers et al., 1993, Yamaga et al., 1996). It can be hypothesised that further increase of dietary mineral supply in peripartal dogs will not prevent excessively increased bone turnover but might cause adverse effects such as reduced availability of other minerals. Moreover, it might be undesirable to prevent bone resorption completely because this might increase the risk for periods with subnormal calcium blood concentrations.

We used the marker of bone formation bALP in this study to be able to compare with own data of non-reproducing adult dogs of the same age, gender and breed as well as with the results Liesegang and Wichert (2012) obtained in peripartal cats. The serum values for bALP decreased from the very high concentrations during late gestation to values still above the reference range in seven out of eight individuals (p = .0035). The decrease in this marker was also observed in cats: Liesegang and Wichert (2012) reported of low concentrations during the last weeks of gestation, which decreased even more during the first weeks of lactation. This difference might be due to the fact that the cats in the study of Liesegang and Wichert (2012) only realised a calcium and phosphorus intake clearly below the recommended daily allowance during late gestation and peak lactation in cats (NRC, 2006) in contrast to a high calcium and phosphorus supply in the dogs of this study and not to a species difference.

Assuming that the reference ranges for crosslaps and bALP are also valid for peripartal dogs, the results of this study would suggest the recommended intake of both minerals according to NRC (2006) suffices. However, the values of both crosslaps and bALP, determined with the same methods, were clearly lower in adult dogs of same breeds and ages during maintenance (Schmitt et al., 2017) indicating an increased bone turnover during the peripartal period. According to Allen (2003), age, diseases, surgery, medication and exercise, respectively, as well as biological variability within the population, might influence bALP. In our trial, most of these factors do not apply. Hence, we were able to demonstrate in this study that there is a sensitivity of bALP during the studied phases of reproduction in dogs. However, other existing markers of bone formation might have been used in addition. The serum concentrations of total and ionised calcium as well as phosphorus did not change and stayed within the reference ranges. The same was true for PTH.

5 | CONCLUSION

Based on our findings especially of the bone markers, which stayed within the reference range during the peripartal phase and did not indicate predominant bone resorption, we hypothesise that the recommendations for the daily supply with calcium and phosphorus in gestating dogs (DLG, 1989; FEDIAF 2018; Meyer et al., 1985; NRC, 2006) can be interpreted as sufficient to meet the requirements during this phase. A further increase of dietary calcium and phosphorus supply in peripartal dogs would probably not prevent a certain physiological increase of bone resorption because (a) adult dogs during maintenance did not adapt to a prolonged feeding of a low calcium diet (Schmitt et al., 2017) and (b) even species which regulate their mineral balance more via intestinal absorption than dogs do, use their skeletal stores during gestation (Bronner & Pansu, 1999; Malm, 1958; McKay et al., 1942; Potgieter, 1940; Walker, 1951).

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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