The effect of metoclopramide treatment on serum prolactin levels, milk composition and yield, and puppy growth rate in bitches during the first week of lactation

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Abstract: Die Auswirkung der Verabreichung von Metoclopramid (MC) auf den Serumprolaktinspiegel (PRL), den Laktosegehalt (LM) sowie die Bruttoenergie (BE) und Trockenmasse der Milch (TM) wurde an 20 gesunden Zuchthündinnen verschiedener Rassen in der Frühlaktation untersucht. LM in der Milch und die Gewichtszunahme der 121 Welpen wurden bestimmt um die Wirkung auf die Milchproduktion abzuschätzen. Zehn Hunde erhielten 6 Tage (T-Gruppe) MC (0,2 mg / kg per os qid), beginnend 10-24 Stunden nach der Geburt des letzten Welpen (Tag 0). Zehn Hündinnen dienten als Kontrollen (C-Gruppe). Blut- und Milchproben wurden bei den Hündinnen am Tag 0,1,2,4 und 6 gesammelt. Die Milchproben von Tag 1 und 2 sowie von Tag 4 und 6 wurden aufgrund des geringen Volumens gepoolt. Die Welpen wurden zweimal täglich gewogen. PRL stieg in beiden Gruppen über die Zeit deutlich an. Mittels Kontrastanalyse zeigte sich ein PRL-Anstieg von Tag 0 auf Tag 1 in der T- ($P = 0{,}050$), nicht aber in der C-Gruppe. LM nahm über die Studienzeit deutlich zu und war in der T-Gruppe höher als in der C-Gruppe. Die TM war unverändert, während sich der Zeitverlauf der BE zwischen C- und T-Hunden unterschied ($P = 0{,}006$). Die Gewichtszunahme der Welpen beider Gruppen war vergleichbar. Bei gesunden Hündinnen induzierte die orale Anwendung von MC post partum eine transiente PRL-Zunahme und stimulierte die Milchlaktoseproduktion. Hunde mit unzureichender oder verzögerter Milchproduktion könnten von dieser Behandlung profitieren.

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The effect of metoclopramide treatment on serum prolactin levels, milk composition and yield, and puppy growth rate in bitches during the first week of lactation

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Für meine treuen Weggefährten

Inhaltsverzeichnis

Zusammenfassung

Die Auswirkung der Verabreichung von Metoclopramid (MC) auf den Serumprolaktinspiegel (PRL), den Laktosegehalt (LM) sowie die Bruttoenergie (BE) und Trockenmasse der Milch (TM) wurde an 20 gesunden Zuchthündinnen verschiedener Rassen in der Frühlaktation untersucht. LM in der Milch und die Gewichtszunahme der 121 Welpen wurden bestimmt um die Wirkung auf die Milchproduktion abzuschätzen. Zehn Hunde erhielten 6 Tage (T-Gruppe) MC (0,2 mg / kg per os qid), beginnend 10-24 Stunden nach der Geburt des letzten Welpen (Tag 0). Zehn Hündinnen dienten als Kontrollen (C-Gruppe). Blut- und Milchproben wurden bei den Hündinnen am Tag 0,1,2,4 und 6 gesammelt. Die Milchproben von Tag 1 und 2 sowie von Tag 4 und 6 wurden aufgrund des geringen Volumens gepoolt. Die Welpen wurden zweimal täglich gewogen. PRL stieg in beiden Gruppen über die Zeit deutlich an. Mittels Kontrastanalyse zeigte sich ein PRL-Anstieg von Tag 0 auf Tag 1 in der T- ($P = 0,050$), nicht aber in der C-Gruppe. LM nahm über die Studienzeit deutlich zu und war in der T-Gruppe höher als in der C-Gruppe. Die TM war unverändert, während sich der Zeitverlauf der BE zwischen C- und T- Hunden unterschied ($P = 0,006$). Die Gewichtszunahme der Welpen beider Gruppen war vergleichbar. Bei gesunden Hündinnen induzierte die orale Anwendung von MC post partum eine transiente PRL-Zunahme und stimulierte die Milchlaktoseproduktion. Hunde mit unzureichender oder verzögerter Milchproduktion könnten von dieser Behandlung profitieren.

Prolaktin, Metoclopramid, Laktosegehalt, Milchmenge

Summary

Twenty client-owned, healthy breeding bitches of various breeds and their 121 pups were used to study the effect of oral metoclopramide (MC) on serum prolactin (PRL) levels, on milk lactose, gross energy and dry matter during early lactation. Milk lactose and puppy weight gain were determined to estimate treatment effect on milk production. Ten dogs received MC (0.2 mg/kg [0.09 mg/lb], per os, every 6 hours) for 6 days (T group) starting 10-24 hours after the birth of the last puppy (Day 0). Ten bitches served as controls (C group). Blood and milk samples from all bitches were collected on Day 0,1,2,4 and 6. Milk samples on Day 1 and 2, and on Day 4 and 6 were pooled due to small volume. Puppies were weighed twice daily. Serum PRL increased significantly over time in both groups, and no treatment effect was seen. When the day-to-day change was analysed with contrasts, PRL increased from Day 0 to Day 1 in the T ($P = 0.050$) but not in the C group. Milk lactose increased significantly during the study period and was higher in the T than in the C group. Milk dry matter was unchanged, while the time course of milk gross energy differed between treated and control dogs ($P = 0.006$). Puppy weight gain was not affected by MC treatment. In healthy bitches, the oral application of MC after parturition induced a transient serum PRL increase and stimulated milk lactose production. Especially dogs with insufficient or delayed milk production could benefit from this treatment effect.

Prolactin, metoclopramide, milk lactose, milk yield

The effect of metoclopramide treatment on serum prolactin levels, milk composition and yield, and on puppy weight gain in bitches during the first week of lactation

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Abstract

Objectives

To investigate the effect of oral metoclopramide (MC) on serum prolactin (PRL) levels, on milk lactose, gross energy and dry matter during early lactation in healthy bitches. Milk lactose and puppy weight gain were determined to estimate treatment effect on milk production.

Animals

Twenty client-owned, healthy breeding bitches of various breeds and their 121 pups.

Procedures

Ten dogs received MC (0.2 mg/kg [0.09 mg/lb], per os, every 6 hours) for 6 days (T group) starting 10-24 hours after the birth of the last puppy (Day 0). Ten bitches served as controls (C group). Blood and milk samples from all bitches were collected on Day 0,1,2,4 and 6. Milk samples on Day 1 and 2, and on Day 4 and 6 were pooled due to small volume. Puppies were weighed twice daily.

Results

Serum PRL increased significantly over time in both groups, and no treatment effect was seen. When the day-to-day change was analyzed with contrasts, PRL increased from Day 0 to Day 1 in the T (P = 0.050) but not in the C group. Milk lactose increased significantly during the study period and was higher in the T than in the C group. Milk dry matter was unchanged, while the time course of milk gross energy differed between treated and control dogs (P = 0.006). Puppy weight gain was not affected by MC treatment.

Conclusions and Clinical Relevance

In healthy bitches, the oral application of MC after parturition induced a transient serum PRL increase and stimulated milk lactose production. Especially dogs with insufficient or delayed milk production could benefit from this treatment effect.

Abbreviations

DM Dry matter
GE Gross energy
MC Metoclopramide
PRL Prolactin
PP Postpartum

Agalactia or hypogalactia in a PP bitch may pose serious risk to the health of the neonates. When puppies do not ingest sufficient amount of colostrum in the first 16-24 hours after birth, failure of passive immune transfer occurs affecting their growth and survival.[1,2] If neonates are not adequately nourished, they are at high risk of fading, becoming septic and may die soon after birth. Primary agalactia or hypogalactia is extremely rare and is associated with anatomical abnormalities of the mammary gland or a lack of response to physiological stimuli.[3] In cases of secondary hypogalactia, milk production is decreased due to concurrent disease of the dam e.g. metritis, mastitis. Furthermore, hypocalcemia, stress (e.g. Cesarean section), premature delivery or undernourishment of the bitch may all lead to insufficient milk yield.[3] Certain drugs or hormones, e.g. slow-release deslorelin implant or cabergoline, administered during pregnancy or lactation may also negatively influence milk production.[4-6]

Prolactin is essential for mammary gland development, initiation and maintenance of lactation.[7-9] It is a polypeptide hormone produced by the lactotroph cells of the anterior pituitary.[10] The most important regulator of PRL secretion is dopamine, which exerts a tonic inhibition from the hypothalamus.[9] PRL secretion is stimulated by several substances and hormones, e.g. estradiol, thyrotrophin-releasing hormone, ocytocin, vasoactive intestinal peptide, serotonin, opioid peptides or angiotensin II.[9,11-13] In the bitch, substantial increases in serum PRL levels occur 16-56 hours prepartum, reaching peak levels on the day of parturition, ~8-32 h before birth.[14,15] After a decrease to near or below prepartum levels in the first 24-48 h following delivery, PRL levels increase again to a secondary peak on Day 10 PP.[14,15] During lactation, high plasma PRL concentrations are present with large variations within and among individuals, [14,15] which may be partly due to the circadian rhythm of PRL. [5] PRL stimulates α-lactalbumin production, [9,16,17] which is the regulatory subunit of the lactose synthase complex within the mammary gland.[18,19] Lactose, through its osmotic actions, is the major determinant of the aqueous phase and thus the volume of milk.[20] Milk lactose increased in Beagle bitches from 3.47% at 7-9 days PP to 4.13% at 29-30 days PP, while none of the other milk constituents varied over time.[21] Similarly, Macias Rostami et al.,[22] and Adkins et al.,[23] also showed lactose in canine milk rising in the first five days of lactation or until two weeks PP, respectively.

Milk lactose concentration and milk volume increased significantly in mothers with PRL deficiency and with lactation insufficiency after administration of recombinant human PRL.[24] Increased PRL levels and galactorrhoea are reported side effects of the administration of MC, which is an antidopaminergic gastrointestinal prokinetic used for the prevention and treatment of nausea and vomiting.[25,26] Therefore, MC is used as a galactogogue in human medicine to increase milk production.[27,28] In puerperal women with term or premature infants and with or without lactational insufficiency, daily doses of 30 or 45 mg MC significantly increased serum PRL and/or milk yield;[29-34] the higher dose resulted in faster onset of effects.[29] However, others reported that the same daily amount (30 mg MC) failed to augment milk production in women with premature newborns.[35,36] In dogs, a single intravenous injection of 0.4 mg/kg [0.18 mg/lb] MC resulted in a significant, transient serum PRL increase in healthy, anestrous bitches,[37] and in male Beagles, MC at 0.2 mg/kg [0.09 mg/lb], per os, every 8 hours was able to significantly stimulate PRL

concentration from 4.5 ± 1.1 ng/ml to 6.5 ± 1.6 ng/ml.[38] This shows that also in dogs, pituitary PRL secretion is modulated by the administration of dopamine D2 receptor antagonists. Although the use of MC to treat agalactia/hypogalactia in bitches on the basis of its PRL-stimulating actions is anecdotal and lacking scientific evidence, there are various protocols recommending MC at dosages ranging from 0.1-0.2 mg/kg [0.045-0.09 mg/lb] or 0.2-0.5 mg/kg [0.09-0.225 mg/lb] every 6-8 or 8-12 hours,[3,39-41] to 1-5 mg/kg [0.45-2.25 mg/lb], every 6-8 hours[40] subcutaneously or orally. The clinical efficacy of these regimens however has not yet been confirmed in a controlled study.

The effects of galactogogues on milk yield and maternal PRL levels are widely studied, but their influence on milk composition is still not well known. In mothers with full-term newborns, MC did not alter milk PRL and sodium concentration,[34] and did not influence total fat, dry matter, fat-free dry matter and total nitrogen content, but promoted the shift from colostrum to mature milk.[30] Domperidone, another antidopaminergic gastrointestinal prokinetic which is also used in human medicine as a galactogogue, increased yield, carbohydrate and calcium content of preterm human milk compared to placebo treatment.[42] The influence of MC administration on milk composition in dogs has not yet been studied.

Our objective was to investigate the effect of MC on serum prolactin levels, milk lactose and milk energy content during early lactation in bitches. Milk lactose and weight gain of the puppies were determined to estimate treatment effect on milk production.

Materials and methods

Animals—Over a 2-year period, 20 client-owned healthy bitches and their 138 healthy pups were included in the study. Written consent for all dogs to participate in the study was obtained from the owners. The study was approved by the Cantonal Veterinary Office of Zurich (permit no. 09/2012). The bitches were assigned into two groups by alternating inclusion into treatment (T; n=10) or control (C; n=10) group at the time of parturition. Only dogs undergoing natural birth and in good clinical condition throughout the study period were included. In the T group, there were 2 Boxers and one of each of the following breeds: Flatcoated Retriever, Labrador Retriever, Labradoodle, Tervueren, Great Dane, Leonberger, Border Collie, Rhodesian Ridgeback. The following dogs belonged to the C group: 4 Boxers, 2

Flatcoated Retrievers, 1 Golden Retriever, 1 German Shepherd, 1 Groenendael and 1 Continental bulldog. During the study, the bitches with their puppies were kept by their owners in their normal environment.

Treatment—Bitches in the T group received 0.2 mg/kg [0.09 mg/lb] MC as an oral suspension[a] every 6 hours for 6 days after parturition. Treatment started between 10 to 24 hours after the birth of the last puppy (Day 0), immediately after the collection of the first blood and milk samples. None of the bitches in this study (T and C groups) received any other medication.

Blood and milk sample collection—The start of the study was defined as Day 0, between 10-24 hours after the birth of the last puppy in a litter. Blood samples (4-5 mL) were collected from the jugular vein into a serum tube on Day 0, 1, 2, 4 and Day 6, twice each day, 30 minutes apart, to account for the pulsatile release of PRL.[43,44] Because peripheral PRL levels in early lactating bitches may also show a circadian rhythm,[5] in each individual bitch, blood samples were collected always at the same time of the day, i.e. morning, afternoon or evening. Cooled blood samples were transported to the clinic and centrifuged for 10 minutes at 3'000 g. Serum was harvested and stored at -80°C until analysis. At the same time of blood sampling, 1-2 mL of milk taken from several glands of each bitch was collected. Day 0 blood and milk samples in the T group were taken before starting the MC treatment. Clinical examinations were carried out by the same veterinarian on each sampling day. Palpation of the mammary glands and macroscopic evaluation of the milk were used to detect early signs for mastitis and to estimate milk yield.

Determination of serum prolactin levels—Serum PRL concentration was analyzed with a previously validated heterologous radioimmunoassay.[45] All samples were run in one batch. The intra-assay coefficient of variation was 3.5%, and the lower limit of detection was 0.8 ng/ml.

Determination of milk composition and gross energy—Milk samples before treatment (Day 0) and after the start of treatment (Day 1-6) were analyzed. Due to insufficient amount of milk on individual days, milk from Day 1 and 2, and from Day 4 and 6 was pooled for the measurements. Defrosted milk was analyzed

for GE using an anisothermal bomb calorimeter[b], and expressed on DM basis (GE/DM; J/g DM). The DM content of milk (%) was determined by drying of 0.1-0.4 g milk at 103°C up to weight constancy. Milk lactose content was determined using a modification of the method previously described.[22] Briefly, whole milk was dissolved 1:1000 (g/g) in distilled water, passed through a 0.22 µm membrane filter to remove particles and transferred to a 2mL autosampler vial. An aliquot (25 µL) was analyzed on a high performance anion exchange liquid chromatograph equipped with a pulsed amperometric detector[c] using a Carbopac PA1 column (4 × 250 mm, Thermo) held at 25 ºC. Lactose was eluted at 1.0 ml/min using a sodium hydroxide/sodium acetate gradient as follows: 0 min, 50 mM NaOH; 2 min, 50 mM NaOH; 18 min, 135 mM NaOH; 18.1 min, 140 mM NaOH + 7.5 mM sodium acetate; 25 min, 140 mM NaOH + 7.5 mM sodium acetate; 35 min, 142.5 mM NaOH + 12.5 mM sodium acetate; 35.1 min, 189 mM NaOH + 90 mM sodium acetate; 45 min, 210 mM NaOH + 100 mM sodium acetate; 45.1 min, 300 mM NaOH + 250 mM sodium acetate; 50 min, 300 mM NaOH + 250 mM sodium acetate; 50.1 min, 50 mM NaOH; 60 min, 50 mM NaOH. Under these conditions lactose has a retention time of approximately 12.5 min. Detection was performed by pulsed amperometry using a gold working electrode and a standard quadruple waveform for carbohydrates (t= 0 s, V=+0.1 V; t = 0.2 s, V=+0.1 V; t= 0.4 s, V= +0.1 V; t= 0.41 s, V= -2.0 V; t= 0.42 s, V= -2.0 V; t = 0.43 s, V=+0.6 V; t= 0.44 s, V= -0.1 V; t =0.50 s, V=-0.1 V). Quantification was performed using an external calibration curve. Lactose content was also expressed on DM milk (Lactose/DM, g/kg).

Puppy weight gain—Body weight of each puppy was recorded by the owners immediately after parturition and twice daily thereafter (morning and evening) during the study period.

Statistical analysis—Age, body weight, body condition score, litter size (live pups) and parity (no. of current pregnancy) of the bitches on Day 0 was compared between the T and C groups by independent samples T-test and Mann-Whitney U test. The mean of the two serum PRL concentrations, which were collected 30 min. apart from each animal on a given day, was used for the statistical calculations. The change in PRL serum levels over time and the effect of treatment, the bitches' age, body weight, parity (primi- or pluriparous) and litter size was analyzed with mixed

models. The day-to-day change of serum PRL concentration in the C and T groups was compared using contrast in the mixed model. Milk composition and milk gross energy were analyzed with a mixed model, and included treatment, the bitches' age, body weight, parity and litter size as independent variables. Because puppy weight recordings on the last study day (Day 6) were not complete, we chose the evening weight on Day 5 as endpoint. The change in puppy weights was therefore analyzed between Day 0 and Day 5 with a mixed model using the bitch as a random factor, and treatment, the bitches' weight, parity, litter size and initial weight of the pups as independent variables. Data was analyzed with computerized statistical programs[d,e]. Significance was set at $P \leq 0.050$.

Results

Animals—The T and C groups were similar in terms of age, body weight, body condition score and litter size (Table 1). There were four and two primiparous, and six and eight pluriparous animals among the T and C bitches, respectively. Current pregnancy number was also not different between the groups (Table 1).

Table 1—General description of the bitches in the control group (n=10) and in the metoclopramide treatment group (n=10) at the start of the study (Day 0*).

Groups	Age (years)	Body weight (kg; lbs)	BCS‡	Litter size (live pups)	Current pregnancy number
Treatment (n=10)	4.4 ± 1.8	31.6 ± 12.9; 69.6 ± 28.4	4.7 ± 0.8 (n=9)	6.9 ± 1.9	2.2 ± 1.4
Control (n=10)	4.3 ± 1.7	27.7 ± 4.9; 61.0 ± 10.8	4.3 ± 0.5 (n=9)	6.9 ± 2.4	2.3 ± 1.2
P value†	0.823	0.580	0.247	1.000	0.684

Values represent the mean ± SD. *Day 0: 10-24 hours after the birth of the last puppy. †P value ≤ 0.050 within a column denotes statistical difference between the treatment and control groups. ‡BCS: body condition score (on a 9-point scale system,[46]).

Serum PRL concentrations—PRL concentration of the 20 bitches at the time of whelping was 26.27 ± 10.1 ng/mL (mean ± SD), and increased significantly over time in both groups from Day 0 to Day 6 ($P = 0.032$ in C, and $P = 0.041$ in T; Figure 1).

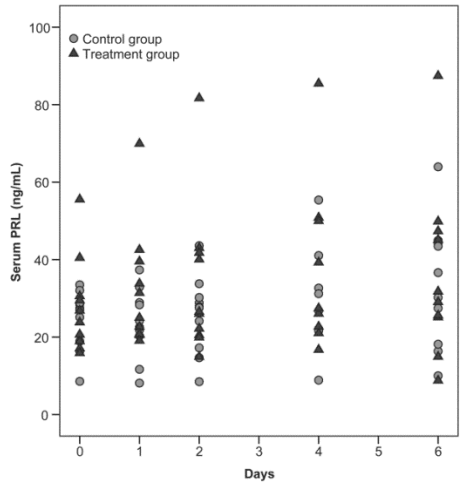

Figure 1—Serum PRL concentrations in the control (n=10) and treatment group (n=10) from Day 0 (after parturition and before the start of treatment) to Day 6 of the study. Treated bitches received MC at 0.2 mg/kg [0.09 mg/lb], per os, every 6 hours, for 6 days.

No effect of treatment on PRL levels was shown in the mixed model (P = 0.087). When the day-to-day change was separately analyzed with contrasts, serum PRL concentrations increased significantly from Day 0 to Day 1 in the T group (P = 0.050), but not in the C group (P = 0.998) (Figure 2).

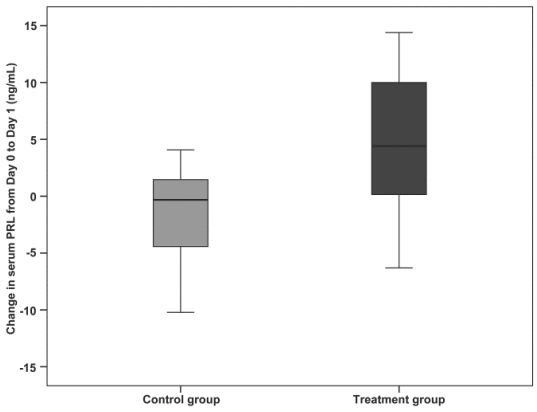

Figure 2—Change in serum PRL concentration from Day 0 (after parturition and before the start of treatment) to Day 1 in the bitches of the control (n=10) and treatment (n=10) group. Treated bitches received MC at 0.2 mg/kg [0.09 mg/lb], per os, every 6 hours, for 6 days.

There was no difference between the groups in the day-to-day change of serum PRL later on (P ≥ 0.171). The bitches' age, body weight, parity and litter size did not influence serum PRL values (P ≥ 0.137). The lowest (8.56 ng/mL) and highest (55.54 ng/mL) PRL concentrations on Day 0 (before the start of treatment)

were found in a Groenendael bitch and in a Rhodesian Ridgeback, respectively. PRL levels in this Ridgeback belonging to the T group were always the highest (55.54-87.43 ng/mL), and in the Groenendael belonging to the C group almost always the lowest (8.12-10.00 ng/mL) of all bitches during the whole study period.

Milk composition—Milk DM content did not significantly change over time (P=0.287), and did not differ between the T and C group (P=0.156) (Figure 3).

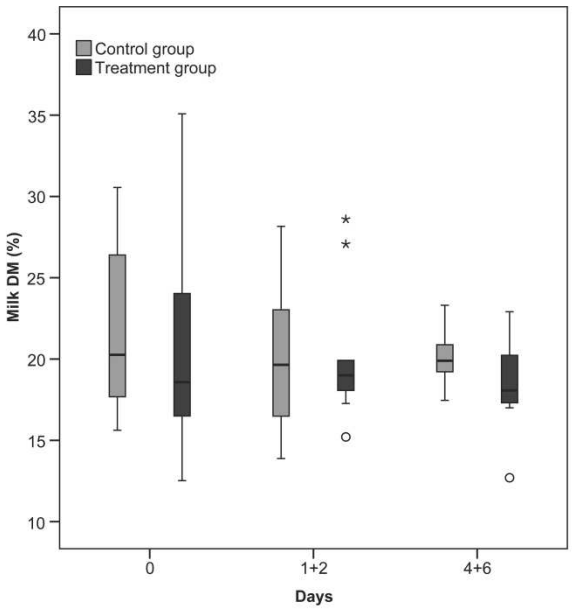

Figure 3—Milk DM (%) in the control (n=10) and MC-treated bitches (n=10) in samples collected on Day 0 (after parturition and before the start of treatment), and in samples pooled from Day 1 and 2, and from Day 4 and 6.

Milk lactose content and Lactose/DM increased significantly over time (P<0.0001). Treated bitches had higher lactose content in milk, both as lactose (g) per kg whole milk (Figure 4A) and as Lactose/DM (Figure 4B) compared to C dogs (P = 0.035 and P = 0.004, respectively).

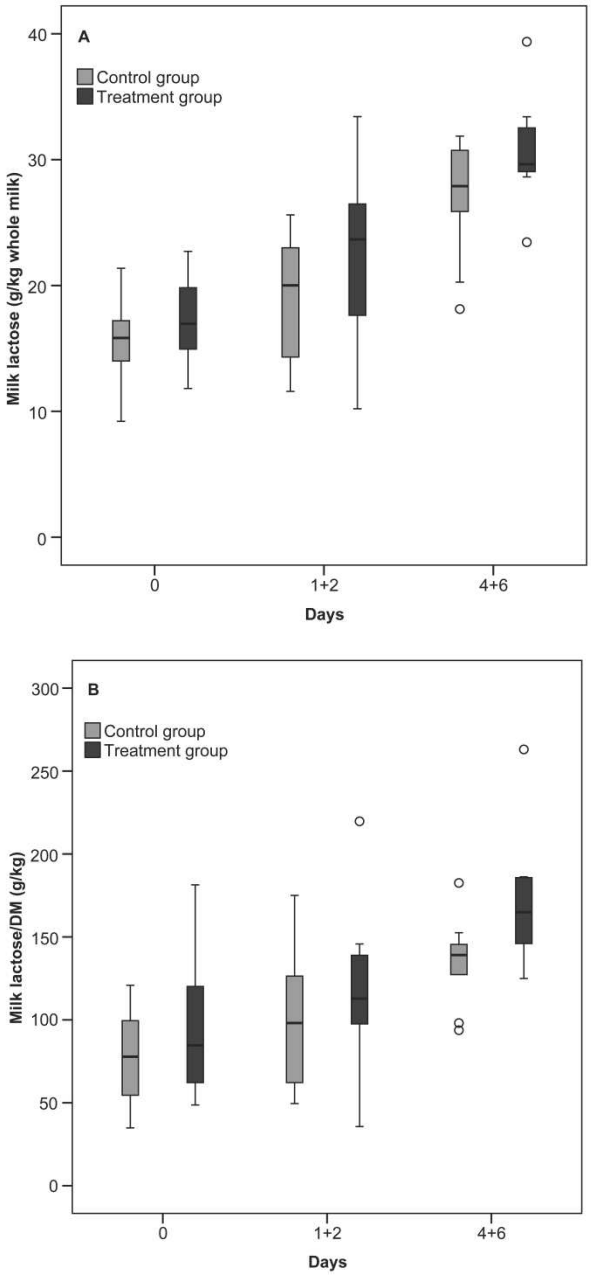

Figure 4—A: Milk lactose content (g/kg whole milk) and B: Lactose/DM (g/kg) in the control (n=10) and MC-treated bitches (n=10) in milk samples collected on Day 0 (after parturition and before the start of treatment), and in samples pooled from Day 1 and 2, and from Day 4 and 6.

Pluriparous bitches had lower milk lactose content (P=0.034) and Lactose/DM (P=0.016) than primiparous dogs. The time course of GE/DM differed significantly between the T and C group (P = 0.006). While in the C animals GE/DM increased steadily, in the T group, after a higher increase from Day 0 to Day 1+2, it decreased by Day 4+6 reaching similar values to the C group (Figure 5). Litter size was inversely related to milk GE/DM (P = 0.016).

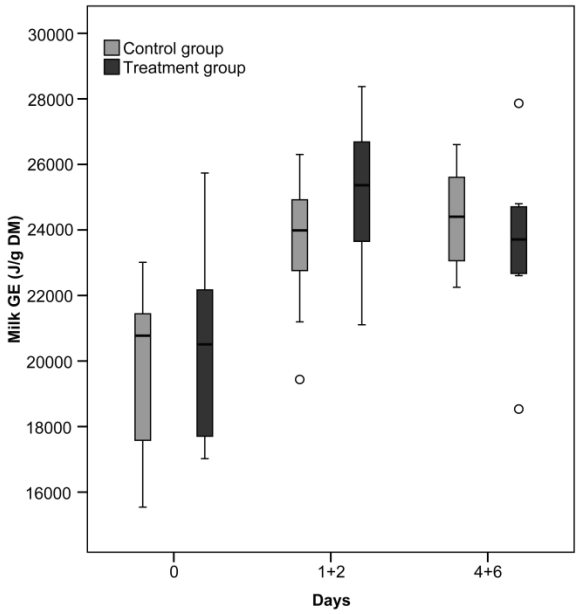

Figure 5—Milk GE expressed on DM basis (J/g DM) in the control (n=10) and MC-treated bitches (n=10) in milk samples collected on Day 0 (after parturition and before the start of treatment), and in samples pooled from Day 1 and 2, and from Day 4 and 6.

Puppy weight gain—The bitches gave birth to a total of 147 puppies, of which 138 were alive at birth and 9 were stillborn. Further two puppies in the C group died during the study period, and two puppies from the T group were excluded because they were fed with milk replacer. Puppy weights (n=13) were not available from two bitches in the T group, and information on neonatal losses during the study was available from only one of them. Overall, data of 121 pups (67 from the C bitches and 54 from the T bitches) were analyzed.

Heavier puppies as well as puppies of heavier bitches gained significantly more weight than smaller pups (Figure 6) or those of smaller dams (P=0.0002 and P=0.032, respectively).

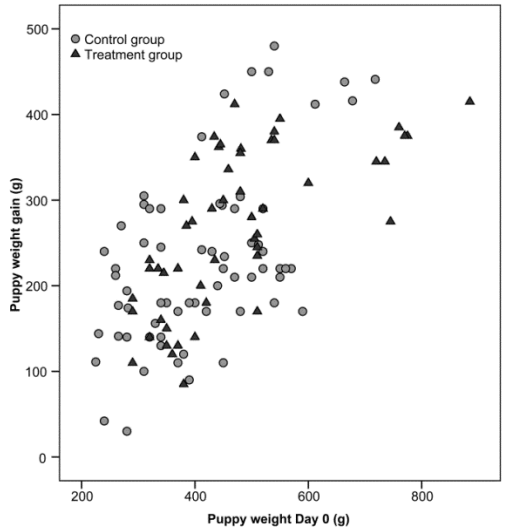

Figure 6—Weight gain of the puppies (n=67 of the control bitches, n=54 of the MC-treated bitches) during the study period (between Day 0 and Day 5) in relation to their starting weight on Day 0.

Litter size seemed to have an inverse relationship with pup weight gain (P=0.055), while parity of the dam had no influence (P=0.792). Treatment did neither affect weight gain of the puppies (P=0.665; Figure 6) nor had any obvious effects on health status or behavior.

Discussion

Despite recommendations in the literature,[3,39,40] the clinical efficacy of the dopamine D2 receptor antagonist MC as a galactogogue has not been evaluated in a controlled study in dogs. The opposite effect of the dopamine D2 receptor agonist cabergoline, i.e. the suppression of PRL secretion and the reduction of milk production in lactating bitches has been known for decades.[5,6] The successful application of domperidone, another dopamine D2 receptor antagonist, was only recently described as a case report in a queen with agalactia.[47] To estimate the effect of MC on milk production, we measured serum prolactin levels, milk lactose, milk energy and puppy weight gain. Over the study period, PRL levels were increasing and reached the highest values in the end of the study, which is in agreement with the results of previous reports.[14,15] Interestingly, the known serum PRL decline of the first 24-48 hours after whelping was seen in most C bitches, while it only occurred in two bitches of the T group. We assume that the administration of MC prevented this initial decrease in serum PRL. This finding would fit to our

hypothesis of MC increasing PRL secretion. From a practical point of view, it would be of great benefit, if the MC-induced PRL increase resulted in increased colostrum production. The most important phase of colostrum intake for the pups is in the first four hours after birth,[2] however, this critical time window could not be covered by our study design. Although this initial PRL increase was seen only in the MC-treated dogs, PRL levels did not differ between the groups later on. The large variation of serum PRL, which was apparent already before treatment and throughout the study, may have masked the effect of treatment in this small number of dogs. This finding is in agreement with the report of Onclin and Verstegen,[15] who also studied the course of PRL concentration in nursing bitches, and assumed that the high variability was caused by the effect of suckling of the pups. Indeed, nursing of the young has been shown to directly stimulate PRL release from the anterior pituitary in several species.[48,49] In rodent studies, the PRL rise in response to nursing has been shown by its positive correlation with litter size, i.e. intensity of the suckling stimulus.[50,51] Although we could have minimized the effect of suckling by separating the pups from the bitches before blood collection, this would have interfered with the goal of the owners to not disturb pup-bitch bonding. We still attempted to diminish possible physiological PRL variations[43,44] by collecting blood twice, 30 min. apart, and calculating the mean of the two values. To account for the possible circadian rhythm of PRL, which was shown in lactating Beagle bitches,[5] we always collected the blood samples in each individual bitch at the same time of the day. However, nursing of the pups in some of the bitches during blood collection as well as a possible circadian variation most likely contributed to the high variability of our PRL data, thereby masking an MC-effect. The same may be true for litter size, which, in contrast to previous reports,[50,51] was not recognized as an influencing factor in our study. However, according to studies in women,[32] continuous oral MC administration eliminate the substantial PRL rise in response to suckling. If this were true in dogs at the low dosage used in our study, nursing would have influenced serum PRL variability only in the C group, but it would have had no or perhaps minimal influence in the T group. However, due to the study design with client-owned dogs, this was not investigated. Furthermore, individual and/or breed differences in serum PRL concentration may exist as shown for German shepherds having lower PRL levels than Beagles during the luteal phase.[52] Although investigating breed variations was not in the scope of our study, it is interesting to note that a Rhodesian Ridgeback

bitch in the T group had PRL levels twice as high as all the other dogs before and after treatment.

Besides PRL as an indirect estimate of milk production, we also measured more direct variables in the milk like lactose, which is known to increase the aqueous phase of milk and thus volume through its osmotic actions.[20] Similarly to the results of previous reports,[21-23] milk lactose levels (on whole milk and on DM basis) increased significantly over time in our study. Because this increase in milk lactose content after parturition seems to coincide with increasing milk yield in the dog[53] as well as in women and in cows,[54,55] the steeper increase of milk lactose levels in the T group may be associated with increased milk production. Determination of milk osmolality could have added more information on the physiological changes during lactation as well as on the pharmaceutical effects of MC, and will be included in a future study. Besides lactose content, we also assessed the effect of MC on milk quality by measuring DM and GE/DM. DM content in the milk was not affected by time or by treatment, but GE/DM was increasing. This increase was noted over time, and interestingly, occurred earlier in the T group. These findings together with increasing Lactose/DM indicate that milk composition was not only changing over time, but that these changes might be initiated earlier by MC treatment. The effects of MC on milk quality implicate that this treatment may increase milk production earlier, which could be of great benefit for hypogalactic bitches. Still, it should be kept in mind that the number of dogs included in our study was small, the measured values were highly variable, and the amount of milk samples was limited. It would have been worth to evaluate milk composition in more details and to start one day before parturition. This would have allowed us to determine whether MC promoted the shift from colostrum to mature milk based on amino acid profile in the bitch, as it does in healthy puerperal women.[30] However, such a study design would have required laboratory dogs, as it is not feasible with client-owned animals.

Although our results on serum PRL and milk composition seem to support the positive influence of MC on milk yield, these measurements cannot substitute the determination of milk output. In women, either emptying the breast by pumping or weighing the infants before and after nursing is used to assess milk production.[29-32] However, emptying all the glands in a dog is not possible. Weighing all the puppies in the litter before and after nursing was included in our study design, but it could not be carried out because of lack of owner compliance. We still recorded all puppy

weights twice daily, and analysed their growth pattern as an indirect measure of the amount of milk produced by the bitch. Pup weight gain was negatively influenced by litter size, which is also known from rats.[56] This can be explained by the reduced energy content of the milk in bitches carrying larger litters. Surprisingly, pluriparous dogs had similar milk DM as primiparous bitches, but lower lactose concentration in milk and Lactose/DM. This does not fit to our clinical experience that pluriparous bitches initiate lactation earlier, and therefore to our expectations of a higher milk lactose content. However, the weight gain of the pups in the two parity groups was similar. We could not show any effect of treatment on pup weight gain, which is not surprising, as treated and control bitches were healthy and without clinical signs of hypogalactia. To prove the positive effect of maternal MC treatment on weight gain, development and health of puppies of hypogalactic bitches, a study with affected dams would be needed. However, to have a control hypogalactia group, which means neither treating the mothers with MC nor supplementing the pups that are not thriving with milk replacer, is not ethical.

The dose of MC used in our study (0.2 mg/kg [0.09 mg/lb], per os, every 6 hours) corresponds to the recommendations in the literature ranging from 0.1-0.5 mg/kg [0.045-0.225 mg/lb] every 6-12 hours[3,39-41] to 1-5 mg/kg [0.45-2.25 mg/lb] every 6-8 hours.[40] The safety of drugs which are not approved for the given indication is always a concern, as it was the case in our study using MC to increase milk production in lactating bitches. In the Plumb's Veterinary Drug Handbook[26] MC is described for the promotion of milk production in dogs at a dose of 0.1–0.2 mg/kg [0.045-0.09 mg/lb], subcutaneously, every 12 hours, without mentioning apparent risk to the nursing offspring. Even though the European Medicines Agency limits the direct application of MC to children, the most commonly reported adverse effects (e.g. extrapyramidal symptoms, diarrhea, sedation) in a recent systematic review and meta-analysis on MC safety in children were reversible and had no long-term significance.[57] In nursing mothers, the use of MC as a galactogogue was recommended, however due to side effects (e.g. headache, diarrhea), domperidone, which does not cross the blood–brain barrier as readily as MC, was recently proposed as a safer alternative.[33] After the start of our study, domperidone has been recommended as a galactogogue in dogs[40] and might be a good or even better alternative to MC, although its clinical efficacy is not confirmed in a controlled study.

In conclusion, in healthy bitches, the oral application of MC at a dose of 0.2 mg/kg [0.09 mg/lb] every 6 hours for 6 days starting immediately after parturition, induced a significant serum PRL increase from day 0 to day 1, and enhanced milk lactose production. Especially dogs with insufficient or delayed milk production could benefit from this effect.

Footnotes

[a]Paspertin®, BGP Products GmbH, Baar, Switzerland

[b]IKA calorimeter C2000 Basic, IKA-Werke GmbH, Staufen, Germany

[c]HPAEC-PAD, ICS3000, Thermo Fischer Dionex, Sunnyvale, CA

[d]IBM® SPSS® Statistics for Windows, Version 22.0, Armonk, NY

[e]R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

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