Effect of metoclopramide treatment of bitches during the first week of lactation on serum prolactin concentration, milk composition, and milk yield and on weight gain of their puppies

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Received March 10, 2017.
Accepted May 19, 2017.

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OBJECTIVE
To investigate effects of metoclopramide orally administered to healthy bitches on serum prolactin and milk lactose concentrations, gross energy, and dry matter content and on puppy weight gain during early lactation.

ANIMALS
20 client-owned bitches and their 121 puppies.

PROCEDURES
10 bitches received metoclopramide (0.2 mg/kg, PO, q 6 h for 6 days; treatment group) starting 10 to 24 hours after birth of the last puppy of the litter (day 0), and 10 bitches served as the control group. Blood and milk samples from all bitches were collected on days 0, 1, 2, 4, and 6. Milk samples for days 1 and 2 and days 4 and 6 were pooled because of small volume. Puppies were weighed twice daily.

RESULTS
Serum prolactin concentration increased significantly over time in both groups, and no treatment effect was detected. When day-to-day changes were analyzed, the prolactin concentration increased from day 0 to day 1 in the treatment group but not in the control group. Milk lactose concentration increased significantly and was higher in the treatment group than in the control group. Milk dry matter content was unchanged, whereas the time course for milk gross energy content differed significantly between treatment and control bitches. Puppy weight gain was not affected by metoclopramide treatment.

CONCLUSIONS AND CLINICAL RELEVANCE
Oral administration of metoclopramide to healthy bitches after parturition induced a transient increase in serum prolactin concentration and stimulated milk lactose production. It is likely bitches with insufficient or delayed milk production could benefit from metoclopramide treatment. (*Am J Vet Res* 2018;79:233–241)

Agalactia or hypogalactia in a postpartum bitch may pose serious risks to health of the neonates. When puppies do not ingest a sufficient amount of colostrum within the first 16 to 24 hours after birth, failure of passive immune transfer occurs, which affects their growth and survival.[1,2] If neonates are not adequately nourished, they are at high risk of fading or developing sepsis, and they may die soon after birth. Primary agalactia or hypogalactia is extremely rare and is associated with anatomic abnormalities of the mammary glands or a lack of response to physiologic stimuli.[3] In cases of secondary hypogalactia, milk production is decreased as a result of concurrent disease of the dam (eg, metritis or mastitis). Furthermore, hypocalcemia, stress (eg, Cesarean section), premature parturition, or undernourishment of a bitch may lead to insufficient milk yield.[3] Certain drugs or hormones (eg, slow-release deslorelin implants or cabergoline) administered during pregnancy or lactation may also negatively influence milk production.[4–6]

ABBREVIATIONS

BCS	Body condition score
NaOAc	Sodium acetate
NaOH	Sodium hydroxide

Prolactin is essential for mammary gland development and initiation and maintenance of lactation.[7–9] It is a polypeptide hormone produced by lactotroph cells of the anterior pituitary gland.[10] The most important regulator of prolactin secretion is dopamine, which is produced by the hypothalamus and exerts tonic inhibition.[9] Prolactin secretion is stimulated by several substances and hormones (eg, estradiol, thyrotrophin-releasing hormone, oxytocin, vasoactive intestinal peptide, serotonin, opioid peptides, and angiotensin II).[9,11–13] In bitches, there is a substantial increase in serum prolactin concentration 16 to 56 hours prior to parturition, which reaches peak concentrations approximately 8 to 32 hours before birth.[14,15] Prolactin

concentrations decrease to concentrations similar to or less than prepartum concentrations during the first 24 to 48 hours after parturition, but then increase again to a secondary peak on day 10 after parturition.[14,15] Plasma prolactin concentrations are high during lactation, with large variations within and among individual bitches,[14,15] which may be partly attributable to the circadian rhythm of prolactin concentrations.[5] Prolactin 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 portion and thus the volume of milk.[20] Milk lactose concentration increased in Beagle bitches from 3.47% at 7 to 9 days after parturition to 4.13% at 29 to 30 days after parturition, but none of the other milk constituents differed over time.[21] Similarly, lactose concentration in canine milk was found to increase in the first 5 days of lactation[22] or until 2 weeks after parturition.[23]

Milk lactose concentration and milk volume increased significantly after administration of recombinant human prolactin to women with prolactin deficiency and lactation insufficiency.[24] Increased prolactin concentrations and galactorrhea are adverse effects reported for the administration of metoclopramide, which is an antidopaminergic gastrointestinal prokinetic used for the prevention and treatment of signs of nausea and vomiting.[25,26] Therefore, metoclopramide is used as a galactogogue in human medicine to increase milk production.[27,28] In puerperal women with term or premature infants and that had or did not have lactational insufficiency, daily doses of 30 or 45 mg of metoclopramide significantly increased serum prolactin concentrations, milk yield, or both[29–34]; the higher dose resulted in a more rapid onset of effects.[29] However, the same daily dose (30 mg of metoclopramide) failed to augment milk production in women with premature newborns in other studies.[35,36] A single injection of metoclopramide (0.4 mg/kg, IV) to healthy anestrous bitches resulted in a significant transient increase in serum prolactin concentrations.[37] Administration of metoclopramide (0.2 mg/kg, PO, q 8 h) to male Beagles significantly increased the mean ± SD prolactin concentration from 4.5 ± 1.1 ng/mL to 6.5 ± 1.6 ng/mL.[38] This indicates that pituitary prolactin secretion in dogs is modulated by the administration of dopamine D_2 receptor antagonists. Although the use of metoclopramide to treat agalactia or hypogalactia in bitches because of its prolactin-stimulating actions is anecdotal and lacking scientific evidence, there are various protocols that recommend metoclopramide at lower dosages ranging from 0.1 to 0.2 mg/kg or 0.2 to 0.5 mg/kg every 6 to 8 or 8 to 12 hours[3,39–41] or at higher dosages from 1 to 5 mg/kg every 6 to 8 hours,[40] administered SC or PO. However, the clinical efficacy of these regimens has not been confirmed in a controlled study.

The effects of galactogogues on milk yield and maternal prolactin concentrations have been widely studied, but their influence on milk composition is not well known. In women with full-term newborns, metoclopramide did not alter milk prolactin and sodium concentrations[34] and did not influence total fat, dry matter, fat-free dry matter, and total nitrogen content of milk, but it did promote the shift from colostrum to mature milk.[30] Domperidone, another antidopaminergic gastrointestinal prokinetic that has been used in humans as a galactogogue, increased yield as well as carbohydrate and calcium content of preterm human milk, compared with results of a placebo treatment.[42] To the authors' knowledge, the influence of metoclopramide administration on milk composition of dogs has not been studied.

The objective of the study reported here was to investigate the effect of metoclopramide on serum prolactin concentrations and milk lactose and energy content in bitches during early lactation. Milk lactose concentration and weight gain of puppies were determined to estimate the treatment effect on milk production.

Materials and Methods

Animals

Twenty client-owned healthy bitches and their 138 healthy puppies were included in the study, which was conducted over a 2-year period. Only dogs with a natural birth and in good clinical condition throughout the study period were included. During the study, the bitches with their puppies were kept by their owners in their home environment.

Bitches were assigned into 2 groups by alternating inclusion into the treatment (n = 10) or control (10) group at the time of parturition. The treatment group comprised 2 Boxers and 1 each of Flat-Coated Retriever, Labrador Retriever, Belgian Tervuren, Great Dane, Leonberger, Border Collie, Rhodesian Ridgeback, and Labrador Retriever–Poodle cross. The control group comprised 4 Boxers, 2 Flat-Coated Retrievers, 1 Golden Retriever, 1 German Shepherd Dog, 1 Belgian Sheepdog, and 1 English Bulldog crossbred. Written consent for participation of all dogs was obtained from the owners. The study was approved by the Cantonal Veterinary Office of Zurich (permit No. 09/2012).

Treatment

Bitches in the treatment group received an oral suspension of metoclopramide[a] (0.2 mg/kg, PO, q 6 h for 6 days). Initial blood and milk samples were collected 10 to 24 hours after birth of the last puppy of the litter (day 0), and treatment was started immediately after collection of those samples. None of the bitches in either group received any other medications.

Collection of blood and milk samples

Two blood samples (4 to 5 mL/sample) were collected at 30-minute intervals from the jugular vein into a serum tube on days 0, 1, 2, 4, and 6; 2 samples were collected daily to account for the pulsatile release of prolactin.[43,44] Because peripheral prolactin concentrations in early lactating bitches may have a circadian rhythm,[5]

blood samples were always collected from each bitch at the same time of day (ie, morning, afternoon, or evening). Blood samples were cooled and transported to our clinic. Samples were centrifuged for 10 minutes at 3,000 × *g*. Serum was harvested and stored at –80°C until analysis. At the time of each daily blood sample collection, a pooled milk sample (total of 1 to 2 mL) was collected from several glands of each bitch.

Clinical examinations were performed by the same investigator (SRK) on each day of sample collection. Palpation of the mammary glands and macroscopic evaluation of the milk were used to detect early signs of mastitis and to estimate milk yield. The BCS (scale, 1 to 9)[45] of each bitch was also determined at that time.

Determination of serum prolactin concentration

Serum prolactin concentration was analyzed by use of a previously validated heterologous radioimmunoassay.[46] All samples were assayed in a single 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 obtained before treatment (day 0) and after the start of treatment (day 1 through 6) were analyzed. Because of an insufficient amount of milk on specific days, milk from days 1 and 2 and from days 4 and 6 was pooled for the measurements. Milk was analyzed for gross energy by use of an anisothermal bomb calorimeter[b] and expressed on a dry-matter basis (J/g of dry matter). Dry-matter content of milk (ie, percentage) was determined by drying 0.1 to 0.4 g of milk at 103°C to constant weight. Milk lactose content was determined by use of a modification of the method described elsewhere.[22] Briefly, milk was dissolved in distilled water (dilution, 1:1,000 [g/g]), passed through a 0.22-µm membrane filter to remove particles, and transferred to a 2-mL autosampler vial. An aliquot (25 µL) was analyzed on a high-performance anion-exchange liquid chromatograph equipped with a pulsed amperometric detector[c] by use of a column[d] packed with a pellicular resin agglomerated with microbeads of latex functionalized with quaternary ammonium (10 µm, 4 × 250 mm, with a total anion exchange capacity of 100 µEq) maintained at 25°C. Lactose was eluted at a rate of 1.0 mL/min by use of a gradient of NaOAc in NaOH as follows: 0 minutes, 50mM NaOH; 2 minutes, 50mM NaOH; 18 minutes, 135mM NaOH; 18.1 minutes, 140mM NaOH plus 7.5mM NaOAc; 25 minutes, 140mM NaOH plus 7.5mM NaOAc; 35 minutes, 142.5mM NaOH plus 12.5mM NaOAc; 35.1 minutes, 189mM NaOH plus 90mM NaOAc; 45 minutes, 210mM NaOH plus 100mM NaOAc; 45.1 minutes, 300mM NaOH plus 250mM NaOAc; 50 minutes, 300mM NaOH plus 250mM NaOAc; 50.1 minutes, 50mM NaOH; and 60 minutes, 50mM NaOH. Lactose has a retention time of approximately 12.5 minutes under these conditions. Detection was performed by use of pulsed amperometry with a gold working electrode and a standard quadruple waveform for carbohydrates as follows: 0 seconds, 0.1 V; 0.2 seconds, 0.1 V (integration on); 0.4 seconds, 0.1 V (integration off); 0.41 seconds, –2.0 V; 0.42 seconds, –2.0 V; 0.43 seconds, 0.6 V; 0.44 seconds, –0.1 V; and 0.50 seconds, –0.1 V. Quantification was performed with an external calibration curve. Lactose content was expressed on a dry-matter basis (g of lactose/dry-matter kg of milk).

Puppy weight gain

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

Statistical analysis

Age, body weight, BCS, litter size (number of liveborn pups), and parity (number of the current pregnancy) of bitches on day 0 was compared between the groups by use of *t* and Mann-Whitney *U* tests. The mean of the 2 serum prolactin concentrations (which were obtained at an interval of 30 minutes) for each bitch on a given day was used for statistical calculations. Change in serum prolactin concentrations over time and the effect of treatment and bitch age, body weight, parity (primiparous or multiparous), and litter size were analyzed with mixed models. Day-to-day change in serum prolactin concentration in the treatment and control groups was analyzed by use of contrasts. Milk composition and milk gross energy content were analyzed with a mixed model, which included treatment and bitch age, body weight, parity, and litter size as independent variables. Because weights recorded on the last study day (day 6) were not available for all puppies, we chose the puppy body weight on the evening of day 5 as the endpoint. Therefore, the change in puppy body weight was compared between days 0 and 5 with a mixed model. That model included bitch as a random factor, and treatment, bitch body weight, parity, litter size, and initial body weight of the puppies were independent variables. Data were analyzed with computerized statistical programs.[e,f] Significance was set at values of $P \leq 0.05$.

Table 1—Mean ± SD values for bitches in the control group (n = 10) and the treatment group (10) at the start of study (day 0*) on the effects of oral metoclopranide administration in healthy bitches.

Variable	Treatment	Control	*P* value†
Age (y)	4.4 ± 1.8	4.3 ± 1.7	0.823
Body weight (kg)	31.6 ± 12.9	27.7 ± 4.9	0.580
BCS‡	4.7 ± 0.8§	4.3 ± 0.5§	0.247
Litter size (No. of liveborn pups)	6.9 ± 1.9	6.9 ± 2.4	1.000
No. of current pregnancy	2.2 ± 1.4	2.3 ± 1.2	0.684

*Day 0 was 10 to 24 hours after birth of the last puppy of the litter; bitches in the treatment group received metoclopramide (0.2 mg/kg, PO, q 6 h for 6 days) beginning on day 0. †Values were considered significant at $P \leq 0.05$. ‡Scored on a 9-point scale.[45] §Represents results for only 9 bitches.

Results

Animals

Treatment and control groups were similar with regard to age, body weight, BCS, and litter size **(Table 1)**. There were 4 and 2 primiparous and 6 and 8 multiparous bitches in the treatment and control groups, respectively. Number of the current pregnancy also did not differ significantly between the groups.

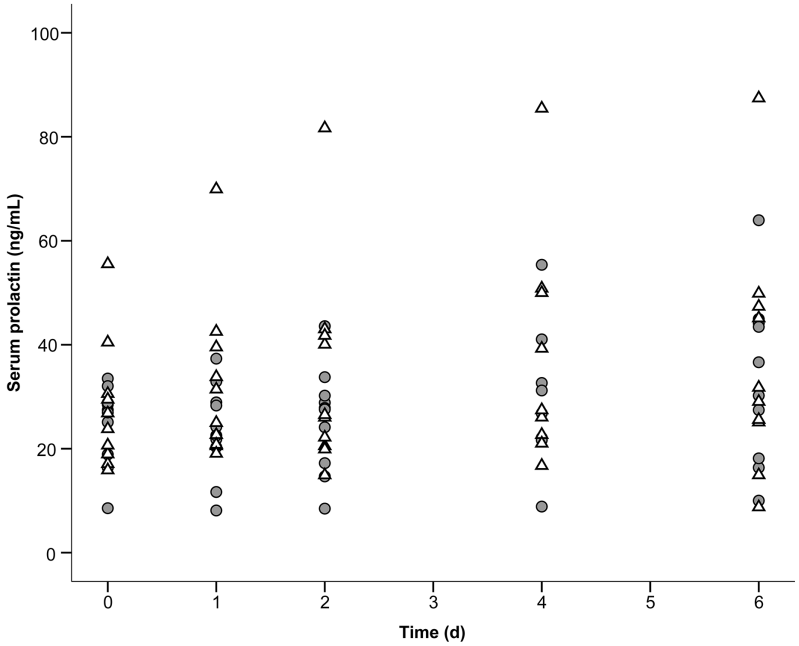

Figure 1—Serum prolactin concentrations in 10 bitches in the control group (gray circles) and 10 bitches in the treatment group (white triangles) from day 0 (10 to 24 hours after birth of the last puppy of the litter) to day 6 in a study of the effects of oral metoclopramide administration in healthy bitches. Bitches in the treatment group received metoclopramide (0.2 mg/kg, PO, q 6 h for 6 days).

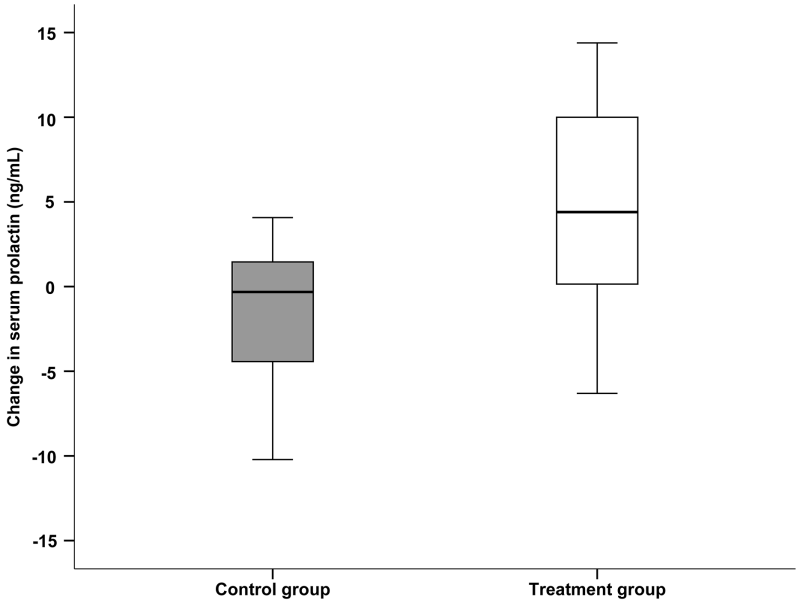

Figure 2—Box-and-whisker plots of the change in serum prolactin concentration from day 0 to day 1 in bitches of the control (n = 10) and treatment (10) groups. Each box represents the first and third quartiles, the horizontal line in each box represents the median value, and the whiskers represent the smallest and largest sample value within 1.5 times the interquartile range. ***See*** Figure 1 for remainder of key.

Serum prolactin concentration

Mean ± SD prolactin concentration of the 20 bitches at the time of whelping was 26.27 ± 10.1 ng/mL, and it increased significantly over time (from day 0 to day 6) in both the control ($P = 0.032$) and treatment ($P = 0.041$) groups **(Figure 1)**. Use of the mixed model revealed no significant ($P = 0.087$) effect of treatment on prolactin concentrations. When change between days was separately analyzed by use of contrasts, serum prolactin concentrations increased significantly from day 0 to day 1 in the treatment group ($P = 0.050$) but not in the control group ($P = 0.998$; **Figure 2**). There was no significant (all $P \geq 0.171$) difference between groups for subsequent day-to-day changes in serum prolactin concentrations. Bitch age, body weight, parity, and litter size did not significantly (all $P \geq 0.137$) affect serum prolactin concentrations. The lowest (8.56 ng/mL) and highest (55.54 ng/mL) prolactin concentrations on day 0 were detected in a Belgian Sheepdog and a Rhodesian Ridgeback, respectively. Prolactin concentrations in the Rhodesian Ridgeback (which was in the treatment group) were always the highest (55.54 to 87.43 ng/mL), and concentrations in the Belgian Sheepdog (which was in the control group) were almost always the lowest (8.12 to 10.00 ng/mL) of all bitches during the study period.

Milk composition

Milk dry-matter content did not change significantly ($P = 0.287$) over time and did not differ significantly ($P = 0.156$) between the treatment and control groups **(Figure 3)**. Milk lactose content and lactose content on a dry-matter basis increased significantly ($P < 0.001$) over time. Treated bitches had significantly higher lactose content in milk, both as grams of lactose per kilogram of milk ($P = 0.035$) and grams of lactose per dry-matter kilogram of milk ($P = 0.004$), compared with lactose content of control dogs **(Figure 4)**. Multiparous bitches had a significantly lower milk lactose content ($P = 0.034$) and lactose content per dry-matter kilogram of milk ($P = 0.016$) than did primiparous dogs. The time course for gross energy on a dry-matter basis differed significantly

($P = 0.006$) between the treatment and control groups. Gross energy on a dry-matter basis increased steadily in the control group, but in the treatment group, there was an initial greater increase from day 0 to days 1 and 2, and it then decreased by days 4 and 6, reaching values similar to the control group **(Figure 5)**. Litter size was significantly ($P = 0.016$) inversely related to milk gross energy on a dry-matter basis.

Puppy weight gain

Bitches gave birth to a total of 147 puppies, of which 138 were liveborn and 9 were stillborn. Two puppies in the control group died during the study period, and 2 puppies in the treatment group were excluded because they were fed milk replacer. Body weight was not available for 13 puppies of 2 bitches in the treatment group, and information on neonatal losses during the study was available for only 1 of those bitches. Overall, data for 121 puppies (67 of control bitches and 54 of treatment bitches) were analyzed.

Puppies with a heavier birthweight as well as puppies of heavier bitches gained significantly more weight than did puppies with a lighter birthweight ($P < 0.001$) and those of lighter bitches ($P = 0.032$; **Figure 6**). Litter size had a nonsignificant ($P = 0.055$) inverse relationship with puppy weight gain, and parity of the bitch did not significantly ($P = 0.792$) affect puppy weight gain. Treatment did not significantly ($P = 0.665$) affect weight gain of the puppies, nor did it have any obvious effects on their health status or their behavior.

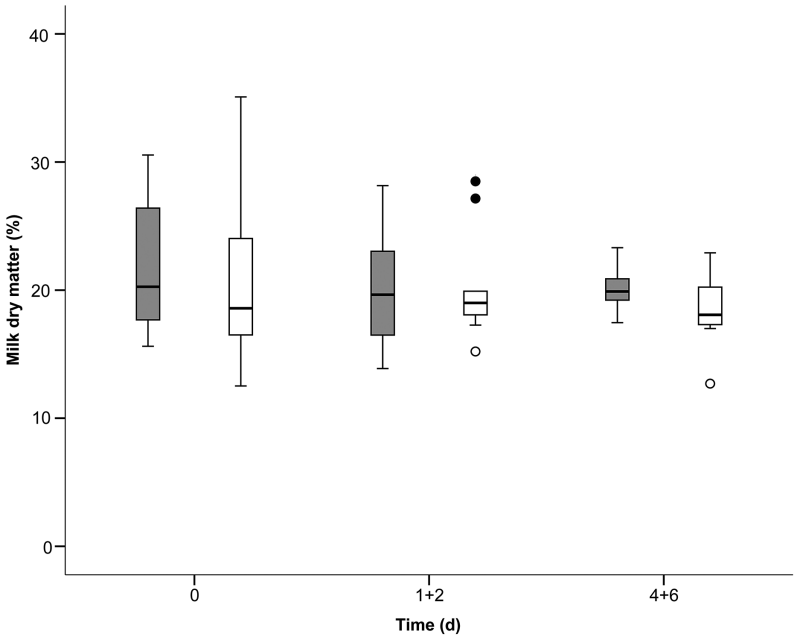

Figure 3—Box-and-whisker plots of milk dry-matter content for bitches in the control (n = 10; gray boxes) and treatment (10; white boxes) groups. Samples were pooled for milk collected on days 1 and 2 and on days 4 and 6. White circles represent sample values between the medium minus 1.5 times the interquartile range and the median minus 3 times the interquartile range, and black circles represent sample values > the median plus 3.0 times the interquartile range. ***See*** Figures 1 and 2 for remainder of key.

Discussion

Despite the fact that there are recommendations for its use in the literature,[3,39,40] the clinical efficacy of the dopamine D_2 receptor antagonist metoclopramide as a galactogogue has not been evaluated in a controlled study in dogs. The opposite effect of the dopamine D_2 receptor agonist cabergoline (ie, suppression of prolactin secretion and reduction of milk production in lactating bitches) has been known for decades.[5,6] The successful application of domperidone, another dopamine D_2 receptor antagonist, was only recently described in a case report of a queen with agalactia.[47] To estimate the effect

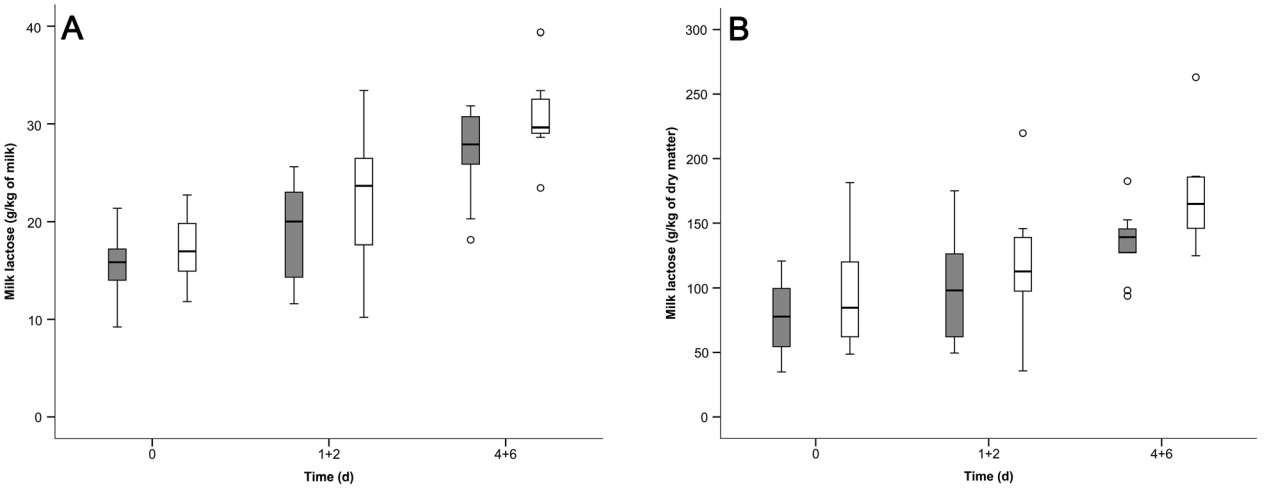

Figure 4—Box-and-whisker plots of milk lactose content (A) and milk lactose content on a dry-matter basis (B) for bitches in the control (n = 10; gray boxes) and treatment (10; white boxes) groups. Samples were pooled for milk collected on days 1 and 2 and on days 4 and 6. ***See*** Figures 1, 2, and 3 for remainder of key.

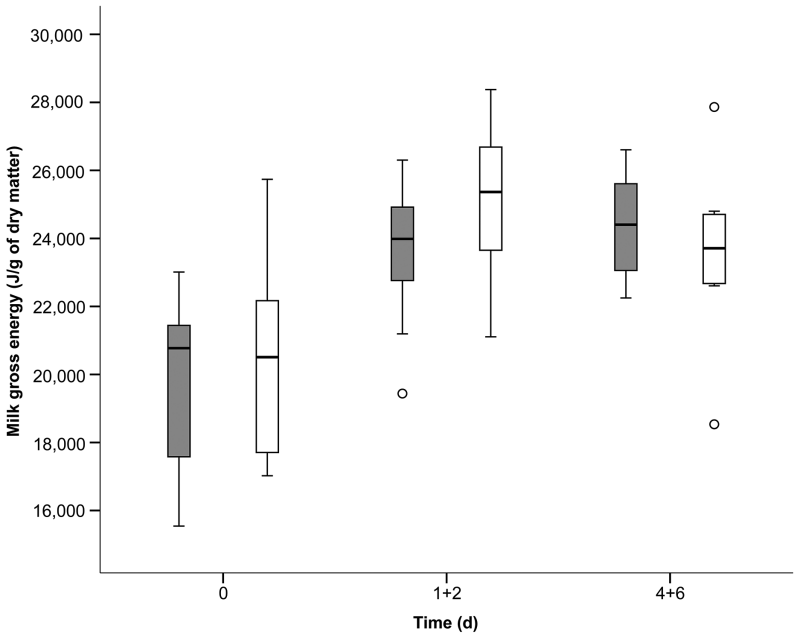

Figure 5—Box-and-whisker plots of gross energy content of milk expressed on a dry-matter basis for bitches in the control (n = 10; gray boxes) and treatment (10; white boxes) groups. Samples were pooled for milk collected on days 1 and 2 and on days 4 and 6. **See** Figures 1, 2, and 3 for remainder of key.

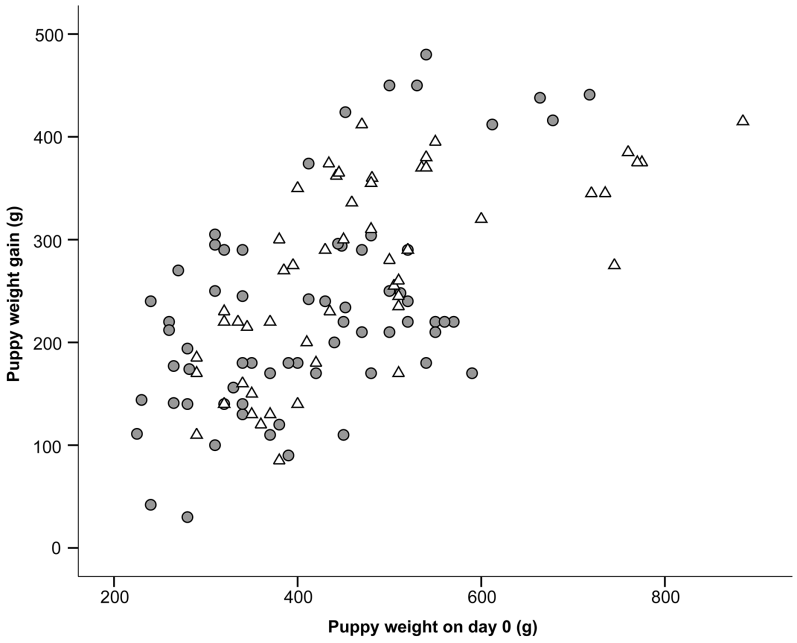

Figure 6—Weight gain of puppies (67 for the control bitches [gray circles] and 54 for the treatment bitches [white triangles]) during the study period (between days 0 and 5) in relation to their starting body weight on day 0.

of metoclopramide on milk production, we measured serum prolactin concentration, milk lactose content, milk energy content, and puppy weight gain. Over the study period, prolactin concentrations increased and reached the highest values at the end of the study, which is in agreement with the results of previous reports.[14,15] Interestingly, the decrease in serum prolactin concentration during the first 24 to 48 hours after whelping was detected in most control bitches, but it was evident in only 2 bitches of the treatment group. We assumed that the administration of metoclopramide prevented this initial decrease in serum prolactin concentration. This finding would fit with our hypothesis that metoclopramide increases prolactin secretion. From a practical point of view, it would be of great benefit if the metoclopramide-induced increase in prolactin concentration resulted in an increase in colostrum production. The most important phase of colostrum intake for puppies is in the first 4 hours after birth[2]; however, this critical time window was not evaluated with our study design. Although this initial increase in prolactin concentration was detected in only the metoclopramide-treated dogs, prolactin concentrations did not differ subsequently between the groups. The large variation in serum prolactin concentrations, which was already apparent before treatment and continued throughout the study, may have masked the effect of treatment in this small number of dogs. This finding is in agreement with that of another report.[15] Investigators of that study[15] evaluated the course of prolactin concentrations in nursing bitches and assumed that the high variability was caused by suckling effects of the puppies. Indeed, nursing of offspring directly stimulates prolactin release from the anterior pituitary gland in several species.[48,49] In rodents, the increase in prolactin concentration in response to suckling is evident as a positive correlation with litter size (ie, intensity of the suckling stimulus).[50,51] Although we could have minimized the effect of suckling by separating the puppies from the bitches before blood collection, this would have interfered with the goal of the owners to not disturb puppy-bitch bonding. We attempted to diminish possible physiologic variations in prolactin concentrations[43,44] by collecting blood samples twice at a 30-minute interval and calculating the mean of the 2 values. To account for the possible circadian rhythm of prolactin concentration, which has been documented in lactating Beagle bitches,[5] blood samples were always collected from each individual bitch at the same time of day. However, suckling of the puppies from some bitches during blood collection as well as a possible circadian varia-

tion most likely contributed to the high variability of the prolactin data, which thereby masked an effect of metoclopramide. The same may have been true for litter size, which, in contrast to previous reports,[50,51] was not an influencing factor in the study reported here. However, on the basis of results for women,[32] continuous oral administration of metoclopramide eliminates the substantial increase in prolactin concentrations in response to suckling. If this were true for dogs at the low dosage used in the present study, suckling would have influenced variability of serum prolactin concentrations in only the control group, but it would have had no or perhaps only a minimal influence in the treatment group. However, because of the study design that involved the use of client-owned dogs, this was not investigated. Furthermore, individual dog or breed differences in serum prolactin concentration may exist; German Shepherd Dogs have a lower prolactin concentration during the luteal phase than do Beagles.[52] Although investigating breed variations was not within the scope of the present study, it was interesting to note that a Rhodesian Ridgeback bitch in the treatment group had prolactin concentrations twice as high as the concentrations of all the other dogs before and after treatment.

In addition to the measurement of prolactin concentrations as an indirect estimate of milk production, we also measured more direct variables such as milk lactose content, which is known to increase the aqueous portion of milk (and thus milk volume) through its osmotic actions.[20] Similar to results of previous reports,[21–23] milk lactose concentrations (on a milk basis and on a dry-matter basis) increased significantly over time in the present study. Because this increase in milk lactose content after parturition appears to coincide with increasing milk yield in dogs[53] as well as in women[54] and cows,[55] the steeper increase in milk lactose concentrations in the treatment group may have been associated with higher milk production. Determination of milk osmolality could have added more information on the physiologic changes during lactation as well as on the pharmaceutical effects of metoclopramide, and this should be included in future studies. In addition to assessment of the lactose content, we also evaluated the effect of metoclopramide on milk quality by measuring dry matter and gross energy on a dry-matter basis. Dry-matter content of the milk was not affected by time or treatment, but gross energy on a dry-matter basis increased. This increase was noted over time and, interestingly, occurred earlier in the treatment group. These findings, together with an increase in lactose content on a dry-matter basis, indicated that milk composition was changing over time and that these changes might be initiated earlier by metoclopramide treatment. Effects of metoclopramide on milk quality indicated that this treatment may have increased milk production earlier, which could be of great benefit for hypogalactic bitches. Still, it must be kept in mind that the number of dogs included in the present study was small, the measured values were highly variable, and the number of milk samples was limited. It would have been worthwhile to evaluate milk composition in more detail and to start collecting samples 1 day before parturition. This would have allowed us to determine whether metoclopramide promoted the shift from colostrum to mature milk on the basis of the amino acid profile in bitches, similar to the effect metoclopramide has in healthy puerperal women.[30] However, such a study design would have required the use of laboratory dogs because it would not be feasible with client-owned animals.

Although our results for serum prolactin concentration and milk composition appeared to support the positive influence of metoclopramide on milk yield, these measurements cannot substitute for the determination of milk output. In women, milk production is assessed by emptying the breasts with a pump or weighing an infant before and after suckling.[29–32] However, emptying all the mammary glands of a bitch is not possible. Weighing all the puppies in the litter before and after suckling was initially included in the study design, but it could not be accomplished because of lack of owner compliance. Puppy body weight was recorded twice daily, and growth pattern of puppies was analyzed as an indirect measure of the amount of milk produced by each bitch. Puppy weight gain was negatively influenced by litter size, which is an effect also seen in rats.[56] This can be explained by the reduced energy content of the milk in bitches with large litters. Surprisingly, multiparous bitches had milk dry-matter content similar to that of primiparous bitches; however, multiparous bitches had a lower lactose concentration in milk and lactose concentration on a dry-matter basis, compared with results of primiparous bitches. This did not fit the authors' clinical experiences that multiparous bitches initiate lactation earlier and, therefore, would be expected to have a higher milk lactose content. However, weight gain of the puppies was similar between the 2 parity groups. There was no effect of treatment on puppy weight gain, which was not surprising, because treatment and control bitches were healthy and did not have clinical signs of hypogalactia. To prove that there is a positive effect of maternal metoclopramide treatment on weight gain, development, and health of puppies of hypogalactic bitches, a study with affected dams would be needed. However, inclusion of a control hypogalactia group, which would involve neither treating the bitches with metoclopramide nor providing supplemental nutrition (ie, milk replacer) to the puppies that are not thriving, is not ethical.

The dose of metoclopramide used in the study reported here (0.2 mg/kg, PO, q 6 h) corresponded with the recommendations in the literature, which range from 0.1 to 0.5 mg/kg every 6 to 12 hours[3,39–41] to 1 to 5 mg/kg every 6 to 8 hours.[40] Safety of drugs that have not been approved for a given indication is always a concern, as was the case in the present

study whereby we administered metoclopramide to increase milk production in lactating bitches. In 1 textbook,[26] metoclopramide (0.1 to 0.2 mg/kg, SC, q 12 h) is described for the promotion of milk production in bitches without any mention of apparent risk to suckling offspring. Although the European Medicines Agency limits the direct application of metoclopramide to children, the most commonly reported adverse effects (eg, extrapyramidal symptoms, diarrhea, and sedation) in a recent systematic review and meta-analysis[57] on metoclopramide safety in children were reversible and had no long-term relevance. In nursing mothers, the use of metoclopramide as a galactogogue has been recommended; however, because of adverse effects (eg, headache and diarrhea), domperidone, which does not cross the blood-brain barrier as readily as metoclopramide, has been proposed as a safer alternative.[33] After we started the study reported here, domperidone was recommended as a galactogogue for bitches[40] and might be a good or even better alternative to metoclopramide, although clinical efficacy of domperidone has not been confirmed in a controlled study.

In the study reported here, oral administration of metoclopramide (0.2 mg/kg, PO, q 6 h) to healthy bitches for 6 days beginning 10 to 24 hours after the end of parturition induced a significant increase in serum prolactin concentration from day 0 to day 1 and enhanced milk lactose production. It is likely that bitches with insufficient or delayed milk production could benefit from this effect.

Acknowledgments

The authors declare that there were no conflicts of interest.

Footnotes

a. Paspertin, BGP Products GmbH, Baar, Switzerland.
b. IKA calorimeter C2000 basic, IKA-Werke GmbH, Staufen, Germany.
c. HPAEC-PAD, ICS3000, Thermo Fisher Scientific, Sunnyvale, Calif.
d. CarboPac PA1, Thermo Fisher Scientific, Sunnyvale, Calif.
e. SPSS Statistics for Windows, version 22.0, IBM Corp, Armonk, NY.
f. R Core Team (2015), version 3.1.3, R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. Available at: www.R-project.org/. Accessed Mar 9, 2015.

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