Effects of Mn supplementation in late-gestating and lactating red deer (*Cervus elaphus hispanicus*) on milk production, milk composition, and calf growth

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ABSTRACT: This study describes the effects of Mn supplementation of 20 late-gestating and lactating Iberian red deer (*Cervus elaphus hispanicus*) females (hinds) fed a balanced diet on milk production and milk composition over the lactation period. Body weight of their calves at birth and at weaning was also evaluated. In addition, the effect of lactation stage was studied. For these purposes, 2 groups of hinds, one composed by 12 individuals (experimental) and the other by 8 individuals (control) were compared. Experimental hinds were s.c. injected weekly with Mn (2 mg Mn/kg BW) from day 140 of gestation until the end of lactation (week 18; forced weaning by physical separation). Control hinds were injected with a physiological saline solution with the same volume and at the same frequency as the experimental group. Serum Mn content of hinds was assessed just before the first Mn injection and at week 10 of lactation to assess whether the injected Mn increased Mn concentrations in blood. No differences were observed

for BW of calves at birth but calves whose mothers were injected with Mn tended ($P = 0.07$) to have greater gain of BW from birth to weaning in proportion of BW at birth compared to calves from control hinds. In addition, supplementation with Mn increased ($P \leq 0.05$) daily milk production by 10.2%, milk fat content by 11.2%, and total fat yield by 17.8%. Also, milk from hinds supplemented with Mn had more Ca ($P < 0.001$) and P ($P < 0.05$) than milk from control hinds. Manganese supplementation did not influence Mn serum content when blood was analyzed at week 10 of lactation, but increased the Mn content of milk by 18.3% ($P < 0.001$). Lactation stage affected ($P < 0.001$) fat, protein, lactose, and DM. Their contents increased as lactation proceeded, and protein was substituted by fat. Therefore, results suggest that Mn supplementation of hinds is recommended, even when they are fed a balanced diet, to increase milk production and the content of fat, Ca, P, and Mn of milk.

Key words: lactation stage, milk nutrient, milk production, Mn supply, offspring growth, red deer

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INTRODUCTION

Calf growth depends on several factors including milk production and composition. Within the composition, literature has focused especially on fat, protein, and lactose to explain the growth (Landete-Castillejos et al., 2001; Kišac et al.,

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2011). However, minerals have also an important role (Gallego et al., 2009). In this respect, benefits obtained with Mn supplementation are well known in dairy cattle for maintaining fertility (NRC, 2007), increasing milk production (Siciliano-Jones et al., 2008; Hackbart et al., 2010), and reducing the incidence of calve health disorders (Teixeira et al., 2014). Despite its importance, in deer there is only information regarding the effects of Mn supply on antler properties (Cappelli et al., 2015), but not on gestating and lactating hinds and, through effects in milk, on their calves. It is important to note that, even when deer are fed a balanced diet, the presence of mineral antagonists in raw materials of diets (Ca, P, or Fe for Mn) may decrease the absorption of Mn producing secondary deficiencies (Henry and Miles, 2000). To avoid this risk, mineral supplementation can be delivered by injection to bypass the gastrointestinal tract.

Trace minerals are essential for fetal development (Hostetler et al., 2003) and the fetus depends completely on the dam for proper supply of these elements (Hidioglou and Knipfel, 1981). If maternal mineral supply is inadequate, fetal development and postnatal performance might be impaired (Marques et al., 2016). Since Mn is transported across the placenta and incorporated into milk, the hypotheses of this research were that supplementation of late-gestating and lactating hinds with this mineral might improve milk production and modify its composition and thus, affect offspring growth.

The aim of this research was to study the effects of Mn supplementation by s.c. injection of late-gestating and lactating hinds fed a balanced diet on milk production and composition and calf growth throughout lactation.

MATERIALS AND METHODS

Ethical Statement

This study was carried out in accordance with the Spanish legislation for the use of animals in research (Boletín Oficial del Estado, 2013) and the approval of the Ethical Committee in Animal Experimentation from the University of Castilla-La Mancha (Permit Number: 1002.04). Handling and sampling were designed to minimize stress and health risks. Hinds and calves were examined daily by farm personnel and weekly by an experienced veterinarian.

Animals and Management

Manganese supplemented ($n = 12$) and control ($n = 8$) hinds (*Cervus elaphus hispanicus*) were kept

in a 5,000-m² open-door enclosure, in captivity conditions similar to those mentioned in García et al. (1999).

Feed and water were offered ad libitum throughout the trial. The feeding program (a concentrate and a total mixed ration) used simulated the feeding system of deer farms, was the same for all hinds, and met or exceeded the nutrient requirements for late pregnancy and lactation of hinds according to NRC (2007). Ingredients and proximate composition (AOAC, 2000) of concentrate and ration are shown in Table 1. Their Mn contents were analyzed by inductively coupled optical emission spectrometry (ICP 6500 DUO Spectrometer/IRIS INTR.EPID II XDL; Thermo Fisher Scientific Inc., Waltham, MA). Calves had free access to the concentrate and to the ration of hinds. The mixed ration was homogenized and cut into small portions in a tractor-driven commercial mixer.

Experimental Design

Experimental hinds were supplied weekly with Mn from day 140 of gestation to day 126 (week 18) of lactation that is the standard lactation period in natural conditions of hinds. As previously used by Cappelli et al. (2015) for deer, Mn was provided by s.c. injections of Mn gluconate (C₁₂H₂₂MnO₁₄, 99.86% purity, Fagron Ibérica S.A.U., Barcelona, Spain). The solution with Mn gluconate was diluted at 8% using distilled water and a sterile and endotoxin-free solution (Aqua B. Braun, B. Braun Melsungen AG, Melsungen, Germany) and contained 2 mg Mn/kg BW (2.5 cm³/100 kg BW). Control group was injected with a physiological saline solution with the same volume and at the same frequency as the experimental group.

Measurements, Sampling, and Analysis

At the beginning of the trial (first Mn injection at day 140 of gestation as average), hinds were assigned to Mn supplementation and control groups, so the average ($\pm$ pooled SEM) age (9.2 ± 1.68 vs. 10.5 ± 2.00 yr), BW (109.5 ± 3.06 vs. 106.3 ± 4.44 kg), and BCS (4.03 ± 0.086 vs. 4.09 ± 0.105) were statistically the same for both groups.

In addition, BW and BCS of hinds were measured 3.5 d before and 3.5 d after calving (± 1 d in both cases) using an electronic balance (± 50 g). The BCS of hinds was measured by palpation of rump, scoring a range from 1 (very poor condition) to 5 (very good condition), with the scale divided in

Table 1. Ingredient and proximate composition of concentrate and total mixed ration (% on DM basis^a)

Item	Concentrate	Total mixed ration
Ingredient composition		
Barley, 10.5% CP	19.2	–
Wheat	7.7	–
Corn	11.3	–
Wheat bran, 20% starch	22.6	–
Barley sprouts	0.9	–
Sunflower meal, 28% CP	13.6	–
Palm kernel expeller	6.3	–
Soybean hulls	11.3	–
Alfalfa meal, 15% CP	11.3	–
Molasses, sugarcane	4.5	–
Grape seed	0.9	–
Palm oil	0.3	–
Calcium carbonate	1.9	–
Salt	0.7	–
Vitamin and mineral premix ^b	0.5	–
Oat	–	27.6
Alfalfa meal, dehydrated	–	48.4
Cereal straw, from barley	–	20.8
Citrus pulp, from orange	–	13.5
Proximate composition		
Moisture	11.6	30.1
CP	15.6	15.6
Ether extract	3.6	1.2
Crude fiber	15.3	34.9
ADF	20.0	38.6
NDF	35.6	57.2
Ash	7.9	10.1
Ca	1.1	1.6
P	0.5	0.2
Mn, ppm	117.8	30.5

^aUnless otherwise indicated.

^bSupplied per kg of concentrate: vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 2,000 IU; vitamin E (all-rac-tocopherol-acetate), 15 mg; Mn (MnSO₄·H₂O), 75 mg; Fe (FeCO₃), 50 mg; Zn (ZnSO₄·H₂O), 115 mg; I (KI), 2 mg; Cu (CuSO₄·5H₂O), 7.5 mg; Se (Na₂SeO₃), 0.22 mg; Co (2CoCO₃·3Co(OH)₂·H₂O), 0.83 mg; ethoxyquin, 0.025 mg; BHT (butylhidrotoluen), 0.18 mg; BHA (butyl-hydroxyanisole), 0.016 mg; sepiolite, 950 mg.

quarters of units, according to what is widely used by deer managers (Audigé et al., 1998; Carrión et al., 2008). Changes of BW and BCS of hinds between consecutive measurements were calculated as indicated by Marques et al. (2016). In addition, hinds were individually weighed (± 50 g) at weeks 2, 4, 6, 10, 14, 16, and 18 of lactation.

Mean calving date (7 males and 5 females) was 16th May $\pm$ 3.4 d in the experimental group and 21st May $\pm$ 2.9 d in the control group (4 males and 4 females). Calves were individually weighed within the first 12 h of life (birth BW) and at 2, 4, 6, 10, 14, 16, and 18 (weaning BW) wk of lactation

using the same electronic balance than for hinds (± 50 g). Data obtained were used to calculate the relative BW gain of calves from birth to weaning at 18 wk of age as indicated by Landete-Castillejos et al. (2001): $[100 \times (\text{weaning BW} - \text{birth BW}) / \text{birth BW}]$.

Hinds were milked using a machine milking setup to 50/50 massage/milking ratio and 44 kPa of vacuum at weeks 2, 4, 6, 10, 14, 16, and 18 of lactation. At the end of the trial, weaning (by physical separation) was enforced simultaneously in all hinds to keep social conditions constant throughout the experiment. During the trial, calves could perform a natural rate of suckling except the days of milking when hinds were separated from their calves for 6 h (0800 to 1400) in a handling facility, as indicated by Landete-Castillejos et al. (2000a, 2000b). Before milking, a low dose of anesthesia was injected in the right jugular vein using 0.5 mg/kg BW of xylazine (Seton 2%, Calier, Barcelona, Spain) and 1 mg/kg BW of ketamine (Imalgene 100, Merial, Lyon, France). After inducing anesthesia, 10 IU of oxytocin (Facilpart, Laboratorios Syva, León, Spain) were injected in the right jugular vein 1 min before milking was started. After milking was finished, anesthesia was reversed with a yohimbine injection (0.25 mg/kg BW; Sigma Chemical Co., St. Louis, MO). Milking frequency was, therefore, reduced to the minimum considered essential to prevent stress and potential damaging effects of the anesthesia. Daily milk production was calculated multiplying by 4 the milk production collected per milking (Landete-Castillejos et al., 2000a, 2000b).

Two milk samples (30 mL each one) were collected from each hind to assess milk composition in duplicate from each milking. Therefore, fat, protein, lactose, and DM concentrations were computed as the mean of the 2 aliquot replicates from each milking. Milk analyses were carried out in an automatic milk analyzer Milkoscan series 4000 (FOSS Analytical A/S, Hillerød, Denmark) based on infrared spectrophotometry. Samples were diluted by 50% with distilled water to adjust their composition to the calibration range of the analyzer. The GE content of fresh milk (GEFM) was calculated as suggested by Landete-Castillejos et al. (2003b). In addition, the total GE produced in milk on a daily basis (total daily GE or TDGE) was calculated multiplying the GEFM by the daily milk production. Data obtained for milk nutrient composition were used to calculate protein:fat ratio (PFR) and total production of milk (total milk yield or TMY), fat (total fat yield or TFY), protein (total protein yield or TPY), lactose (total lactose

yield or **TLY**), and DM (total DM yield or **TDMY**) during lactation. One sample collected at 2, 4, 6, 10, 14, and 18 weeks of lactation was frozen (-20°C) for subsequent mineral analysis.

To assess Mn content in serum, blood samples (5 cc) were taken from resting hinds after an overnight of fast just before the first injection with Mn (basal level) and at week 10 of lactation. Blood samples were drawn, without sedation, by jugular venipuncture (Vacutest Clot activators, Kima S.A., Arzergrande, Italy) and were coagulated in the tubes. Serum was separated using a centrifugal separator ($1,372 \times g$ at 4°C for 15 min) and samples were frozen (-20°C) for subsequent analysis of Mn content. Mineral content of milk and Mn content of serum were analyzed by inductively coupled optical emission spectrometry (ICP 6500 DUO Spectrometer/IRIS INTR.EPID II XDL; Thermo Fisher Scientific Inc., Waltham, MA).

Statistical Analysis

Prerequisites to run each test were checked and data normality was tested with the Shapiro–Wilk test. All data were normally distributed. A GLM analysis was performed to study the effects of Mn supplementation on hind BW and BCS (day of the first Mn injection and 3.5 ± 1 d before and after calving), BW of calves, BW (hinds and calves) and BCS (hinds) changes between consecutive measurements, relative BW gain of calves from birth to weaning, TMY, TFY, TPY, TLY, TDMY, and serum Mn content. The Mn content of samples collected at the beginning of the trial (basal level) was used as covariable to analyze the Mn content of serum at week 10 of lactation. Calf gender was not controlled in the experimental design because hinds were assigned to groups at gestation stage before knowing the sex of their fetus. For this reason, all calf variables were analyzed using calf sex as an independent covariate, whereas the treatment $\times$ sex interaction was not tested because the experimental units were not blocked by calf sex. Nevertheless, sex was not a significant ($P > 0.10$) covariate for weaning variables (BW at weaning, BW change from birth to weaning, and relative BW gain). In addition, calf birth BW was used as covariable to analyze BW at weaning but was not significant ($P > 0.10$) and, in consequence, was removed from the analysis. Furthermore, a GLM analysis was performed to study the sex effects of offspring in milk production and milk composition. Global data (including those from Mn supplemented and control groups) were used because no significant

interactions between sex $\times$ Mn supplementation were detected.

On the other hand, a MIXED procedure was used to study the effects of Mn supplementation, using lactation stage (week) as a repeated effect and hind as the subject. The effects of Mn supplementation, hind within treatment, lactation stage, and Mn supplementation $\times$ lactation stage interaction were tested for BW of hind and calves throughout lactation, daily milk production, milk composition (fat, protein, PFR, lactose, DM, and mineral content), GEFM, and TDGE. When the model was significant for lactation stage, a Tukey test was used to make pairwise comparison among means. In addition, the effect of hind BW on milk production and other pairs of variables were analyzed using bilateral Pearson correlations.

In all cases, the replicate was the animal. Data in tables are presented as means for all traits and analyses were carried out with SPSS version 19 (SPSS Inc., Chicago, IL). Significance was set at $P < 0.05$, and trends were considered as marginally significant if $P \geq 0.05$ and $P < 0.10$.

RESULTS

No significant interactions between Mn supplementation and lactation stage were detected for any variable measured. Therefore, only main effects are shown.

Effects of Mn Supplementation on Hind and Calf Traits Studied

Manganese supplementation did not influence BW or BCS of hinds at any stage studied, nor BW of calves at birth (Table 2). No differences were observed between treatments in absolute calf gains (mean $\pm$ SEM Mn group vs. mean $\pm$ SEM control group) during lactation. However, when weaning BW was corrected taking account the variability in birth BW (relative BW gain of calves), calves from hinds that were supplemented with Mn tended ($P = 0.07$) to have a greater BW gain from birth to weaning than calves from control hinds. In addition, supplementation with Mn increased ($P \leq 0.05$) 10.2% daily milk production, 11.2% fat content, 17.8% TFY, 4.2% DM, 5.9% GEFM, and 15.6% TDGE (Table 3 and Fig. 1). However, Mn supplementation did not influence protein and lactose content of milk, TMY, TPY, TLY, and TDMY. Therefore, PFR was greater ($P < 0.001$) for milk from control hinds than for milk from Mn supplemented hinds.

On the other hand, supplementation with Mn increased ($P < 0.05$) the content of Ca, P, Mg, Sr, and Rb and tended ($P = 0.05$) to increase the Cr content of milk (Table 4). However, milk from hinds supplemented with Mn had less ($P = 0.02$) Na content and tended ($P = 0.06$) to have less Cu content than milk from control hinds. Despite Mn supplementation increased the content of this mineral in milk ($P < 0.001$), no differences (mean $\pm$ pooled SEM) were observed for the Mn content of serum at week 10 of lactation (0.16 ± 0.007 and 0.15 ± 0.007 ppm for Mn injected and control groups, respectively; $P = 0.31$).

Effects of Lactation Stage on Hind and Calf Traits Studied

Hind BW did not change throughout lactation. However, daily milk production decreased continuously during lactation ($P < 0.001$) from 3,158 mL/d at week 2 to 1,611 mL/d at week 18. These decrease in milk production did not remain stable during lactation and was greatest at the beginning (24.2%,

week 2 to 6) and at the end of the study period (23.6%, week 14 to 18) than between weeks 10 and 14 (8.5%).

Fat averaged 9.4% over the 18-wk period and increased ($P < 0.001$) with lactation stage from 7.9% to 12.4%. Milk protein averaged 7.0% for the 18-wk period and, also increased ($P < 0.001$) with stage of lactation from 6.6% to 7.6% between weeks 2 and 18. The PFR averaged 0.77 for the 18-wk period and was affected ($P < 0.001$) by lactation stage; protein was substituted by fat over the lactation period. Lactose content, with an average value of 4.9% for the 18 weeks of lactation, increased throughout lactation ($P < 0.001$). Content of DM, as expected, showed a similar trend to that for fat and protein ($P < 0.001$) and achieved a mean value of 23.3% for the 18-wk period. On the other hand, GEFM (1,556 kcal/kg as average) increased ($P < 0.001$) and TDGE (3,610 kcal/d as average) decreased ($P < 0.001$) throughout the lactation.

Milk mineral composition also varied with lactation stage. The highest content of Ca was observed at weeks 2, 14, and 18 of lactation ($P < 0.001$). Milk had more P at week 18 of lactation than between weeks 4 and 14, with values at week 2 being intermediates ($P < 0.001$, see Table 4). Milk collected between weeks 2 and 10 had more K than milk collected between weeks 14 and 18 ($P < 0.001$). Milk Mn content was greater ($P = 0.02$) at weeks 2 and 18 than at weeks 4 to 14. Milk Zn content increased throughout the lactation ($P = 0.001$).

Effects of Calf Sex on Production and Composition of Milk

Milk for males had greater content than milk for females in Ca (0.214 ± 0.0033 vs. 0.207 ± 0.0032 g/100 g; $P = 0.04$), Mg (0.018 ± 0.0003 vs. 0.017 ± 0.0002 g/100 g; $P = 0.003$), Mn (0.99 ± 0.022 vs. 0.93 ± 0.022 mg/kg; $P = 0.04$), Sr (3.50 ± 0.089 vs. 2.96 ± 0.063 mg/kg; $P < 0.001$), and marginally P (0.020 ± 0.0030 vs. 0.019 ± 0.0037 g/100 g; $P = 0.08$) and Zn (12.6 ± 0.38 vs. 11.7 ± 0.43 mg/kg; $P = 0.05$). No sex effect was observed for daily milk production or major milk composition (protein, fat, lactose, and DM content). Finally, males were heavier ($P < 0.001$) than females at birth (9.0 ± 0.07 vs. 8.3 ± 0.11 kg) and at weaning (48.0 ± 0.55 vs. 42.8 ± 0.45 kg).

Correlation Coefficients Among Milk Components and Production Variables

Correlation coefficients were computed among milk components and production variables

Table 2. Effects of Mn treatment^a to hinds on performance of hinds and calves from red deer (mean $\pm$ pooled SEM)^b

Item	Mn treatment	Control	P-value
Hind age, yr	9.2 $\pm$ 1.68	10.5 $\pm$ 2.00	0.62
Hind BW, kg			
First Mn injection ^c	109.5 $\pm$ 3.06	106.3 $\pm$ 4.44	0.56
Before calving ^d	122.5 $\pm$ 3.13	121.2 $\pm$ 4.02	0.81
BW change ^e	13.0 $\pm$ 1.23	14.9 $\pm$ 1.43	0.34
After calving ^d	106.6 $\pm$ 3.10	103.8 $\pm$ 4.16	0.59
BW change ^f	-15.9 $\pm$ 1.26	-17.5 $\pm$ 0.83	0.37
Hind BCS			
First Mn injection ^c	4.03 $\pm$ 0.086	4.09 $\pm$ 0.105	0.64
Before calving ^d	3.85 $\pm$ 0.095	4.09 $\pm$ 0.094	0.10
BCS change ^e	-0.18 $\pm$ 0.116	0.00 $\pm$ 0.106	0.31
After calving ^d	3.83 $\pm$ 0.121	4.00 $\pm$ 0.094	0.33
BCS change ^f	-0.02 $\pm$ 0.136	-0.09 $\pm$ 0.046	0.68
Calf BW, kg			
Birth	8.57 $\pm$ 0.213	8.83 $\pm$ 0.346	0.49
Weaning	46.8 $\pm$ 1.57	43.8 $\pm$ 1.37	0.79
BW change ^g	38.2 $\pm$ 1.51	35.0 $\pm$ 1.22	0.14
Calf relative BW gain, % ^{g,h}	448.2 $\pm$ 17.47	398.7 $\pm$ 17.11	0.07

^aProvided by s.c. injections containing 2.5 cm³/100 kg BW.

^bThe experimental unit was the animal ($n = 12$ for Mn treatment and $n = 8$ for control group).

^cBeginning of the trial at day 140 of gestation.

^d3.5 d before and 3.5 d after calving (± 1 d in both cases).

^eBetween before of calving and the first Mn injection.

^fBetween after and before of calving.

^gBetween weaning and birth.

^h[100 $\times$ (weaning BW - birth BW)/birth BW].

Table 3. Effects of Mn treatment^a to hinds and week of lactation (WL) on hind and calf BW throughout lactation, daily milk production (DMP), milk composition, GE content of fresh milk (GEFM), and total daily GE produced (TDGE) of red deer (mean $\pm$ pooled SEM)^b

Item	Mn treatment		Week of lactation								P-value ^c	
	Mn	Control	2	4	6	10	14	16	18	Mn	WL	
Hind BW, kg	107.6 $\pm$ 1.19	105.0 $\pm$ 1.35	105.1 $\pm$ 2.29	106.6 $\pm$ 2.32	107.0 $\pm$ 2.43	108.3 $\pm$ 2.62	107.9 $\pm$ 2.38	108.3 $\pm$ 2.66	103.3 $\pm$ 2.12	0.18	0.69	
Calf BW, kg	30.5 $\pm$ 1.46	29.1 $\pm$ 1.54	13.1 ^e $\pm$ 0.38	17.9 ^c $\pm$ 0.41	22.5 ^d $\pm$ 0.47	31.2 ^c $\pm$ 0.71	39.6 ^b $\pm$ 1.01	43.1 ^a $\pm$ 1.31	45.6 ^a $\pm$ 1.11	0.61	<0.001	
DMP, mL/d	2,475 $\pm$ 92.5	2,223 $\pm$ 99.5	3,158 ^a $\pm$ 238.5	2,888 ^a $\pm$ 145.2	2,395 ^b $\pm$ 118.5	2,303 ^b $\pm$ 135.5	2,108 ^b $\pm$ 124.8	2,103 ^b $\pm$ 144.1	1,611 ^c $\pm$ 74.1	0.01	<0.001	
Fat, %	9.8 $\pm$ 0.24	8.7 $\pm$ 0.28	7.9 ^c $\pm$ 0.22	8.0 ^c $\pm$ 0.39	7.7 ^c $\pm$ 0.16	8.6 ^c $\pm$ 0.26	10.5 ^b $\pm$ 0.31	11.0 ^b $\pm$ 0.28	12.4 ^a $\pm$ 0.43	<0.001	<0.001	
Protein, %	7.0 $\pm$ 0.06	7.0 $\pm$ 0.10	6.6 ^{cd} $\pm$ 0.05	6.5 ^d $\pm$ 0.08	6.9 ^{bc} $\pm$ 0.10	7.1 ^b $\pm$ 0.19	7.2 ^b $\pm$ 0.10	7.2 ^b $\pm$ 0.10	7.6 ^a $\pm$ 0.13	0.58	<0.001	
Protein:fat	0.75 $\pm$ 0.015	0.83 $\pm$ 0.017	0.85 ^b $\pm$ 0.021	0.84 ^b $\pm$ 0.029	0.91 ^a $\pm$ 0.021	0.83 ^b $\pm$ 0.016	0.70 ^c $\pm$ 0.022	0.66 ^{cd} $\pm$ 0.016	0.63 ^d $\pm$ 0.019	<0.001	<0.001	
Lactose, %	4.9 $\pm$ 0.05	4.9 $\pm$ 0.07	4.8 ^{abc} $\pm$ 0.06	5.0 ^{ab} $\pm$ 0.05	5.1 ^{ab} $\pm$ 0.06	5.1 ^{ab} $\pm$ 0.17	4.6 ^c $\pm$ 0.09	4.6 ^c $\pm$ 0.14	5.3 ^a $\pm$ 0.08	0.51	<0.001	
DM, %	23.6 $\pm$ 0.23	22.6 $\pm$ 0.28	21.7 ^d $\pm$ 0.20	21.7 ^d $\pm$ 0.37	21.7 ^d $\pm$ 0.25	22.8 ^c $\pm$ 0.19	24.3 ^b $\pm$ 0.36	24.7 ^b $\pm$ 0.34	26.0 ^a $\pm$ 0.40	<0.001	<0.001	
GEFM, kcal/kg	1,587 $\pm$ 0.02	1,493 $\pm$ 0.03	1,401 ^c $\pm$ 0.02	1,406 ^c $\pm$ 0.04	1,396 ^c $\pm$ 0.02	1,484 ^c $\pm$ 0.03	1,658 ^b $\pm$ 0.03	1,699 ^b $\pm$ 0.03	1,847 ^a $\pm$ 0.04	<0.001	<0.001	
TDGE, kcal/d	3,848 $\pm$ 0.13	3,247 $\pm$ 0.13	4,445 ^a $\pm$ 0.35	4,059 ^{ab} $\pm$ 0.23	3,341 ^{bc} $\pm$ 0.17	3,436 ^{bc} $\pm$ 0.22	3,478 ^{bc} $\pm$ 0.20	3,560 ^{bc} $\pm$ 0.25	2,950 ^c $\pm$ 0.123	<0.001	<0.001	

^aProvided by s.c. injections containing 2.5 cm³/100 kg BW.^bThe experimental unit was the animal ($n = 20$ per week).^cStatistical analyses took into account the effects of Mn and WL because the interaction Mn $\times$ WL was not significant for all traits studied.^{a-b}Means within a row with different superscripts differ ($P < 0.05$). **Figure 1.** Effects of Mn treatment to hinds on total yields of milk, fat, protein, lactose, and DM in red deer (mean $\pm$ pooled SEM). The experimental unit was the animal ($n = 12$ for Mn treatment and $n = 8$ for control group).

(Tables 5 and 6). A negative relationship between daily milk production and fat content was observed ($r = -0.423$; $P < 0.001$). Dry matter showed an obvious positive correlation ($P < 0.001$) with fat ($r = 0.950$) and protein ($r = 0.656$). In addition, GEFM was positively correlated ($P < 0.001$) with fat ($r = 0.992$), protein ($r = 0.712$), and DM ($r = 0.954$), while correlation with lactose content did not reach significance.

Correlations between mean milk mineral content and daily milk production show an inverse relationship with S ($r = -0.273$; $P = 0.003$) and Al ($r = -0.353$; $P < 0.001$), and a positive correlation with K ($r = 0.385$; $P < 0.001$), but no correlations with Ca, P, Na, or Zn were observed. In addition, several significant correlations were observed between the minerals of milk.

DISCUSSION

Studies about the effects of mineral supplementation in red deer are focused mainly on antler development and antler bone tissue characteristics (Cappelli et al., 2015; Gambín et al., 2017) or internal bones (Olguín et al., 2013). In fact, Cappelli et al. (2015) concluded that Mn supplementation improved structure and some mechanical properties of antlers despite animals having a balanced diet. Authors assessed the influence of mineral supplementation (as salt licks) in milk production and composition (Ceacero et al., 2009; Malacarne et al., 2015) and in calf performance (Gallego et al., 2009) in red deer. However, to our knowledge, there is no any study in the literature involving a fixed dose of one injected mineral (Mn as in this case) in late-gestating and lactating hinds fed with a balanced diet that met or exceeded the nutrient requirements for late pregnancy and lactation of hinds according to NRC (2007). L'Abbé et al. (2003) suggested that

Table 4. Effects of Mn treatment^a to hinds and week of lactation (WL) on milk mineral composition of red deer (mean $\pm$ pooled SEM)^b

Item	Mn treatment		Week of lactation							P-value ^c	
	Mn	Control	2	4	6	10	14	18	Mn	WL	
Macromineral, %											
Ca	0.22 ± 0.003	0.20 ± 0.004	0.22 ^a ± 0.006	0.20 ^b ± 0.004	0.20 ^b ± 0.004	0.20 ^b ± 0.005	0.23 ^a ± 0.006	0.23 ^a ± 0.005	<0.001	<0.001	
P	0.20 ± 0.003	0.19 ± 0.004	0.21 ^b ± 0.004	0.19 ^c ± 0.003	0.19 ^c ± 0.004	0.19 ^c ± 0.005	0.18 ^c ± 0.006	0.22 ^a ± 0.007	0.02	<0.001	
Mg	0.017 ± 0.0002	0.016 ± 0.0003	0.018 ^a ± 0.0004	0.017 ^b ± 0.0004	0.016 ^c ± 0.0004	0.017 ^b ± 0.0005	0.017 ^b ± 0.0005	0.018 ^a ± 0.0005	0.01	0.01	
Na	0.031 ± 0.0005	0.033 ± 0.0006	0.034 ± 0.0011	0.031 ± 0.0009	0.031 ± 0.0008	0.031 ± 0.0010	0.032 ± 0.0008	0.033 ± 0.0010	0.02	0.13	
S	0.052 ± 0.0009	0.052 ± 0.0013	0.052 ^b ± 0.0013	0.045 ^c ± 0.0008	0.047 ^c ± 0.0012	0.052 ^b ± 0.0017	0.054 ^b ± 0.0015	0.063 ^a ± 0.0014	0.90	<0.001	
K	0.15 ± 0.003	0.14 ± 0.003	0.16 ^a ± 0.005	0.16 ^a ± 0.003	0.16 ^a ± 0.004	0.15 ^a ± 0.004	0.13 ^b ± 0.003	0.12 ^b ± 0.002	0.43	<0.001	
Trace mineral, ppm											
B	0.23 ± 0.005	0.23 ± 0.006	0.24 ^{bc} ± 0.009	0.23 ^{bc} ± 0.009	0.25 ^c ± 0.009	0.25 ^c ± 0.008	0.22 ^{ab} ± 0.010	0.20 ^a ± 0.005	0.39	<0.001	
Cu	0.16 ± 0.012	0.18 ± 0.015	0.20 ± 0.023	0.13 ± 0.012	0.17 ± 0.028	0.14 ± 0.020	0.15 ± 0.018	0.21 ± 0.033	0.06	0.06	
Fe	0.46 ± 0.021	0.43 ± 0.023	0.61 ^a ± 0.051	0.37 ^c ± 0.020	0.33 ^c ± 0.015	0.35 ^c ± 0.017	0.48 ^b ± 0.034	0.54 ^{ab} ± 0.031	0.18	<0.001	
Mn	1.04 ± 0.014	0.85 ± 0.014	1.01 ^a ± 0.032	0.92 ^b ± 0.026	0.94 ^b ± 0.037	0.95 ^b ± 0.028	0.96 ^b ± 0.035	1.01 ^a ± 0.032	<0.001	0.02	
Sr	3.4 ± 0.08	3.0 ± 0.09	3.6 ^c ± 0.16	3.7 ^c ± 0.14	3.4 ^{bc} ± 0.16	3.1 ^{ab} ± 0.10	3.0 ^{ab} ± 0.12	2.9 ^a ± 0.12	<0.001	<0.001	
Al	0.81 ± 0.031	0.82 ± 0.037	0.82 ^{bc} ± 0.055	0.63 ^d ± 0.018	0.71 ^{ab} ± 0.031	0.73 ^{ab} ± 0.040	0.95 ^{cd} ± 0.067	1.06 ^a ± 0.065	0.91	<0.001	
Li	0.026 ± 0.0010	0.024 ± 0.0013	0.022 ^c ± 0.0019	0.022 ^c ± 0.0019	0.024 ^{bc} ± 0.0023	0.026 ^{abc} ± 0.0016	0.031 ^a ± 0.0016	0.029 ^{ab} ± 0.0019	0.15	0.01	
Cr	0.03 ± 0.0005	0.02 ± 0.0007	0.03 ^a ± 0.010	0.02 ^b ± 0.0009	0.02 ^b ± 0.010	0.02 ^b ± 0.0006	0.02 ^b ± 0.0013	0.02 ^{ab} ± 0.0006	0.05	<0.001	
Rb	0.83 ± 0.019	0.77 ± 0.022	0.90 ^a ± 0.041	0.82 ^a ± 0.036	0.83 ^a ± 0.030	0.87 ^a ± 0.031	0.71 ^b ± 0.030	0.71 ^b ± 0.032	0.007	0.001	
Zn	11.6 ± 0.31	13.0 ± 0.54	12.7 ^{bc} ± 0.92	10.9 ^c ± 0.39	10.6 ^c ± 0.38	11.8 ^{bc} ± 0.53	12.9 ^{ab} ± 0.75	14.4 ^a ± 0.80	0.03	0.001	

^aProvided by s.c. injections containing 2.5 cm³/100 kg BW.^bThe experimental unit was the animal (n = 12 for Mn treatment and n = 8 for control group).^cStatistical analyses took into account the effects of Mn and WL because the interaction Mn $\times$ WL was not significant for all traits studied.^{a-c}Means within a row with different superscripts differ (P < 0.05).

Table 5. Correlation coefficients between daily milk production (DMP) and milk constituents of red deer (only significal results are shown)

Item	DMP	Hind BW BC ^a	Hind BW AC ^b	Hind BCS BC ^a	Hind BCS AC ^b	Calf birth BW	Calf weaning BW	Fat	Protein	Lactose	DM
Hind BW BC ^a	0.236**										
Hind BW AC ^b	0.334***	0.940***									
Hind BCS BC ^a		0.276***	0.272***								
Hind BCS AC ^b	-0.145 ^{0.09}	0.540***	0.359***	0.272***							
Calf birth BW		-0.468***	-0.515***		-0.198*						
Calf weaning BW		-0.243**	-0.170 ^{0.05}		-0.181*	0.333***					
Fat	-0.423***										
Protein	-0.340***	-0.178*					0.189*	0.617***			
Lactose		0.160 ^{0.06}							0.276***		
DM	-0.426***			-0.146 ^{0.09}				0.950***	0.656***		
GEFM ^c	-0.432***							0.992***	0.712***		0.954***

^aBefore calving.^bAfter calving.^cGE of fresh milk.* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Numerical values have been used for trends ($0.05 \leq P \leq 0.10$).

mineral supplementation above standard level of reference established for mammals could produce beneficial effects.

Current results showed that Mn supplementation of late-gestating and lactating hinds did not affect BW of calves at birth but tended to produce calves with a greater total gain of BW during lactation (corrected by birth BW). Regarding the effect during gestation, the lack of effect for calf birth BW is in agreement with the findings reported by [Stanton et al. \(2000\)](#) and [Sprinkle et al. \(2006\)](#) who supplemented late-gestating beef cows with trace minerals (in organic or inorganic form) and found that this did not impact calf birth BW, despite the fact that many minerals, like Mn, are transported across the placenta. However, some positive effect should be expected because Mn plays an important role on the animal growth, particularly in the synthesis of protein and cartilage mucopolysaccharide and in the metabolism related with the energy, lipids, and vitamins ([Suttle, 2010](#)). In fact, we found a positive effect of Mn on the greater relative BW gains from birth to weaning. Such effect may have 2 causes that are not mutually exclusive: 1) a direct effect of the greater content of Mn in milk from supplemented mothers, as was observed in the current trial; and 2) an indirect effect because supplementing with Mn improves the production of

milk nutrients (fat, Ca, and P), and these, in turn, usually affect calf growth. Thus, milk from hinds supplemented with Mn had 11.2% more fat (17.8% greater TFY) than milk from control hinds. The increase of fat content of milk from Mn supplemented hinds could be through its role as cofactor of the acetyl-coA carboxylase phosphatase enzyme that is implicated in the lipid biosynthetic pathway ([Thampy and Wakil, 1985](#)). In addition, milk from hinds supplemented with Mn had a greater content of some minerals such as Ca, P, and Mn than milk obtained from control hinds. [Gallego et al. \(2009\)](#) concluded that milk minerals (such as Ca, P, and Fe) explained more than a third of the total variability explained by the model for calf growth. Therefore, the increase of fat, Ca, and P content of milk observed in the current trial could be the cause for the greater growth rate of calves from Mn supplemented hinds. One of the mechanisms of the observed effect may be that Mn may increase the growth of some tissues of the body of calves (e.g., skeleton) which, in turn, would prompt the calves to demand higher milk production by the mother, and this would end up in changes in milk production (in fact, it increased 10.2% with Mn supplementation in the current trial) and fat content. Fat is the milk component that can be more easily mobilized in support of lactation ([Landete-Castillejos et al.,](#)

Table 6. Correlation coefficients between daily milk production (DMP) and milk mineral content of red deer (only significant results are shown)

Item	DMP	Ca	P	Mg	Na	S	K	B	Cu	Fe	Mn	Sr	Al	Li	Cr	Rb
Ca		1														
P		0.480***	1													
Mg		0.448***	0.669***	1												
Na		0.229*	0.396***	0.356***	1											
S	-0.273**	0.635***	0.680***	0.566***	0.383***	1										
K	0.385***	-0.412***				-0.515***	1									
B	0.296***	-0.294***	-0.201*	-0.248**	-0.285**	-0.359***	0.470***	1								
Cu			0.233**	0.222*	0.313***	0.222*	-0.334***	-0.365***	0.207*	1						
Fe		0.387***	0.364***	0.358***	0.305***	0.433***	-0.334***	-0.174 ^{0.06}	0.395***	1						
Mn		0.493***	0.571***	0.665***	0.226**	0.322***	0.344***	0.249**	0.333***	0.333***	1					
Sr	0.400***			0.294***		-0.188*	0.344***	0.249**		0.680***	0.240**	1				
Al	-0.353***	0.442***	0.354***	0.336***	0.630***	0.630***	-0.546***	-0.333***			0.300***	-0.282**	1			
Li	-0.219*	0.460***	0.188*	0.351***	0.167 ^{0.07}	0.409***	-0.218*				0.320***		0.341***	1		
Cr	0.251**	0.395***	0.328***	0.261**	0.321***	0.155**				0.404***					1	
Rb	0.301***	-0.229**				-0.275**	0.611***	0.434***					-0.361***	-0.246**		1
Zn		0.193*	0.156 ^{0.09}			0.398***	-0.482***	-0.184 ^{0.05}	0.316***	0.368***	-0.223*		0.454***			-0.293***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Numerical values have been used for trends ($0.05 \leq P \leq 0.10$).

2003a) in contrast to protein (Kaufmann, 1979; Landete-Castillejos et al., 2003a). Current results agree with the increase of TFY observed in cows after trace mineral supplementation including Mn (Ballantine et al., 2002; Griffiths et al., 2007) without any effect on TPY (Griffiths et al., 2007; Siciliano-Jones et al., 2008).

The increment in Ca and P content of milk from the supplemented group was not expected because, in general, the content of macrominerals in milk remains rather stable (McDowell, 2003; Gallego et al., 2006). Calcium content of milk might be near its maximum possible, and its concentration is important in maintaining the transfer of Ca to calf (Gallego et al., 2006). The Ca supply is very important because a reduction in transfer may affect Ca content in calf bones, and small changes in such content can produce large changes in bone mechanical properties (Currey, 2003). Thus, it is very surprising that such concentration in milk varies with Mn supplementation. However, authors previously observed that Mn supplementation increased the content of Ca and P in antlers from adult deer (Landete-Castillejos et al., 2007; Cappelli et al., 2015). Bone turnover is increased during lactation resulting in a mobilization of 5% to 10% of Ca from bone to milk (Kovacs, 2005). Therefore, the increase of Ca and P content of milk with Mn supplementation could be the result of an increase of the bone turnover to deliver the increased content of these minerals in milk.

The increase of Mn content in milk after its supplementation, as occurred in the current trial, has been observed previously in milk from humans (Vuori et al., 1980) and cows (Archibald and Lindquist, 1943). In fact, Vuori et al. (1980) found a positive correlation between Mn consumption and the content of this mineral in milk. Transition element cations (such as Cu, Zn, or Mn) have concentrations in blood, tissues, and milk that are largely independent on their intake (Pechová et al., 2008). In fact, their concentrations are related with the regulation of the gut absorption and vary with the metabolic demands (Windisch, 2002). Therefore, supplementation by injected minerals should result in their increase in milk content, as we found in the current study. However, no differences were observed in serum for Mn content at week 10 of lactation. In consequence, serum concentration of Mn did not reflect the level of Mn supplementation. These results agree with those from Legleiter et al. (2005). The reason could be that Mn is captured quickly from blood by liver. In fact, liver excretion can be increased up to 200 times in response to a

high level of Mn serum in cows (Hall and Symonds, 1981). Thus, the increase of Mn excreted by milk of supplemented hinds would be achieved by fast transfer from the liver or tissue storing Mn achieved at a constant blood serum.

A moderate variability in milk composition in *C. elaphus* has been reported in the literature. Values obtained in the current research were lowest than those reported in the literature for fat, protein, and DM (Arman et al., 1974; Krzywinski et al., 1980; Loudon et al., 1984; Landete-Castillejos et al., 2000b; Malacarne et al., 2015). However, the lactose content observed in the current hinds were higher than that reported by Arman et al. (1974) and by Krzywinski et al. (1980), but lower than reported by Landete-Castillejos et al. (2000b) for 34 weeks of lactation and by Landete-Castillejos et al. (2005) for 91 lactations of a long-term study. Differences in deer milk composition among authors might be caused by inter-study differences in factors affecting milk such as major nutrients in diet and, in particular, its mineral content or applied mineral supplementation, or by body variables and age of the hinds (Landete-Castillejos et al., 2003a, 2005), food availability (Landete-Castillejos et al., 2003a), birth date (Landete-Castillejos et al., 2000b, 2001, 2005), but also by stage of lactation (Landete-Castillejos et al., 2000b; Vergara et al., 2003; Gallego et al., 2006) and sex of the calf (Landete-Castillejos et al., 2005; Gallego et al., 2009).

Respect to stage of lactation, no significant interactions with Mn supplementation were observed in the current trial and results obtained were similar to those obtained previously in deer by our own group (García et al., 1999; Landete-Castillejos et al., 2000b; Vergara et al., 2003; Gallego et al., 2006) and by other research groups (Krzywinski et al., 1980; Malacarne et al., 2015). Regarding sex of calves, milk for male calves had different content in several minerals compared with that of milk for female calves. In particular, milk for male calves had significant greater content of Ca, Mg, Mn, Sr, and marginally P and Zn than milk for female calves. Despite the small sample size, sex effects in milk mineral composition had already been reported in our herd previously (Gallego et al., 2009), although no sex effects were observed for daily milk production or major milk composition (protein, fat, lactose, and DM content), in contrast to studies involving a large sample size (91 lactations; Landete-Castillejos et al., 2005). Results observed for variation of milk mineral profile with offspring sex might be the response to a greater nutrient demand by heavier calves. In fact, in the

current research, males were 8% and 11% heavier than females at birth and weaning, respectively.

Even under ad libitum access to high quality food, mineral supplementation of lactating hinds results in positive benefits (Ceacero et al., 2009). Together with the current results, and because previous studies from our own group showed positive effects of Mn supplementation on composition and mechanical performance of antlers from adult males (Cappelli et al., 2015), Mn supplementation should be recommended for stags and hinds in deer farms. Given that supplementation with Mn (as tested in the current trial), costs only around \$10.00 per individual lactation, Mn injections may increase profitability in deer based on differences observed in the current trial (greater daily milk production and milk with more fat, Ca, P, and Mn). However, it should be noted that, some additional factors not included in this cost (i.e., the amount of labor associated with this type of activity, the associated stress of handling and its impact on production) should be evaluated to determine the profitability of Mn supplementation on red deer.

In conclusion, hinds supplemented weekly with Mn injections in the late-gestation and throughout lactation until 18 weeks increased, even under a balanced diet, 10% their milk production, 11% their milk fat content, and 18% their TFY. Therefore, Mn supplementation is recommended, based on current results, during the late-gestation and during lactation in deer hinds to increase the milk production and the fat content of milk.

Conflict of interest statement. None declared.

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