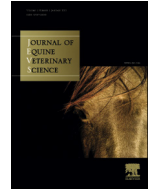




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Original Research

Feeding Magnesium Supplement to Foals Reduces Osteochondrosis Prevalence

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ABSTRACT

The influence of supplements containing magnesium on the etiology of osteochondrosis (OC) is unknown. We did two studies to measure the effect of additional minerals (especially magnesium) on OC. In study 1 (five studs, in total 64 mares and foals aged 0 to 5 months, equally divided into two groups), supplementation with minerals and placebo was used. Blood samples were taken from foals at age of 2, 8, and 16 weeks. At the same time, milk samples were taken from the mare. Bone biomarkers (osteocalcin and C-terminal telopeptide [CTX] of type I [CTX-1] collagen) and minerals (calcium, phosphorus, and magnesium) were measured in blood and the same minerals in milk of the mare. At the end of the study, the femoropatellar (knee), tarsocrural (hock), and metacarpophalangeal and/or metatarsophalangeal (fetlock) were radiographed and scored for the presence and grade of osteochondrotic lesions. In study 2 (six studs, 54 foals aged 5 to 12 months, equally divided into two groups), the same was repeated. At the start and end of the study, again blood samples were taken and analyzed on the same parameters as in study 1. Also, the same radiography was done. In study 1 in the mineral supplemented group, 21.9% were diagnosed with osteochondrosis compared with 41.9% in the placebo group. In study 2, there was no change in OC between 5 and 12 months in the placebo group whereas there was a drop of 14.3% in incidence in the supplement group. We concluded that magnesium supplementation reduced OC prevalence.

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1. Introduction

Osteochondrosis (OC) is a disorder frequently diagnosed in horses. Osteochondrosis prevalence is very high: a prevalence of 25%–40% is no exception in warmblood breeds [1,2], although coldblood horses also suffer from this disorder [2–4]. Osteochondrosis is a disturbance in the process of ossification that occurs in young animals. It is a dynamic disorder and lesions may repair or get worse during the first months until aged 12 months [5,6]. It starts at birth or possibly even before. At an age of 5 months, prevalence is at its highest. Regression of lesions is joint

dependent, but no further substantial reduction in OC is observed after an age of 12 months [6,7].

Several factors do influence bone formation, and irregular ossification leads to the formation of loose fragments. Irrespective of a good genetic background, bone development depends on minerals such as calcium, phosphorus, and magnesium, trace elements such as copper, zinc, and manganese, and vitamins such as vitamin D and K. Previous studies mainly focused on copper, zinc, and other trace elements, but the role of magnesium has not been studied so far [2,8]. This study focuses on the effect of supplementing magnesium and phosphorus during the age of first 12 months of a foal on the development of OC. The second aim of this study was to evaluate the use of previously described biomarkers, osteocalcin and C-terminal telopeptide (CTX) of type I (CTX-1) collagen [4,9–16], as indicators for the risk of OC.

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2. Materials and Method

2.1. Study 1

Sixty-four mares living at five different stud farms were selected for this study. Blood was taken from mares 2 weeks before the calculated day of parturition. Three mares had already given birth to their foal at the start of the experiment. From the 64 foals, one foal died because of a bacterial infection before the end of the experiment at week 16 after parturition. Until they reached the age of 16 weeks, foals were given 42 g per day of an oral paste containing 4.05 g of magnesium and 2.50 g of phosphorus. The placebo group received the same oral paste without magnesium and phosphorus. The pastes were coded red and blue and no further difference in taste or labels existed. The mineral content of the feed was recorded to correct for differences between stud farms. Blood samples were taken from the mares at the beginning of the experiment, and levels of minerals and bone biomarkers were measured. At 2, 8, and 16 weeks after parturition, milk samples from mares and blood samples from foals were collected.

2.2. Study 2

Fifty-four foals living at six stud farms were selected for this study. Foals were randomly divided into two groups per stud farm. One group received 200-g pellets with 4 g of magnesium, 2.5 g of phosphorus, and 1.7 g of calcium. The second group (placebo) received no pellets. Whether foals received extra pellets was only known by the owner of the foals and the supervisor of the stud farms, but not to the researchers. The mineral content of the feed was routinely analyzed by BLGG, Wageningen, The Netherlands (Laboratory specialized in analyzing feed) and recorded to correct for differences between stud farms.

From the 54 foals, two died because of infections not related to this study.

In both studies, blood was collected in the morning from the jugular vein into plain tubes and sent to the laboratory as soon as possible and/or directly afterward. After centrifugation (15 minutes at 2000 g), serum was stored at -20°C until analyses were performed.

Milk samples taken in study 1 were stored in plain tubes and sent to the laboratory together with the blood samples.

Milk samples were analyzed for calcium, magnesium, and phosphorus after 10-fold dilution with water, using inductively coupled plasma optical emission spectrometry (ICP-OES). In a previous experiment, we validated this method with two other methods: after acid digestion in a microwave and detection with ICP-OES and after precipitation of protein and detection with ICP-OES. All three methods gave the same results in our laboratory.

Blood samples were analyzed for calcium, magnesium, and phosphorus, using ICP-OES.

Bone biomarkers were analyzed as follows: C-telopeptide type 1 was analyzed with an enzyme-linked immunosorbent assay (ELISA)-test kit (Immunodiagnostic Systems Inc, Scottsdale, AZ). Osteocalcin was analyzed using an ELISA-test kit (Quidel, Metra Quidel MicroVue 8002, San Diego, CA). Both ELISA kits were validated for use in horses. Linearity, stability, repeatability and within-laboratory reproducibility, accuracy, and selectivity were tested [13,17].

The following parameters were noted for each stud farm: (1) Management: use of chemicals, metals used for construction of stables, drinking system, feeding system, air ventilation, and amount of light in stables; (2) Feeding of mares: hours spent in pasture, feeding additional concentrates, control of feeding amount, and quality of grass; (3) Feeding of foals, additional to milk. (4) Water: origin of water source, control of water supply; (5) Physical exercise: from hours of movement of foals, inside or outside, and space opportunities during night, relative movement was calculated on a scale from 0 (lowest) to 3 (more movement possible for the foal); and (6) From each foal in the second study, the growth was recorded by measuring the wither height at 5 and 12 months.

Osteochondrosis was diagnosed using X-ray at an age of 5 and 12 months according to Van Grevenhof et al [1].

In short, status was assessed using radiographs from eight joints: tarsocrural (TC), femoropatellar (FP), metacarpophalangeal (MCP), and metatarsophalangeal (MTP) joints. A total of 28 predilection sites per animal were scrutinized for the presence of OC lesions. At each site, OC was scored on a categorical scale from A to E (A, normal; E,

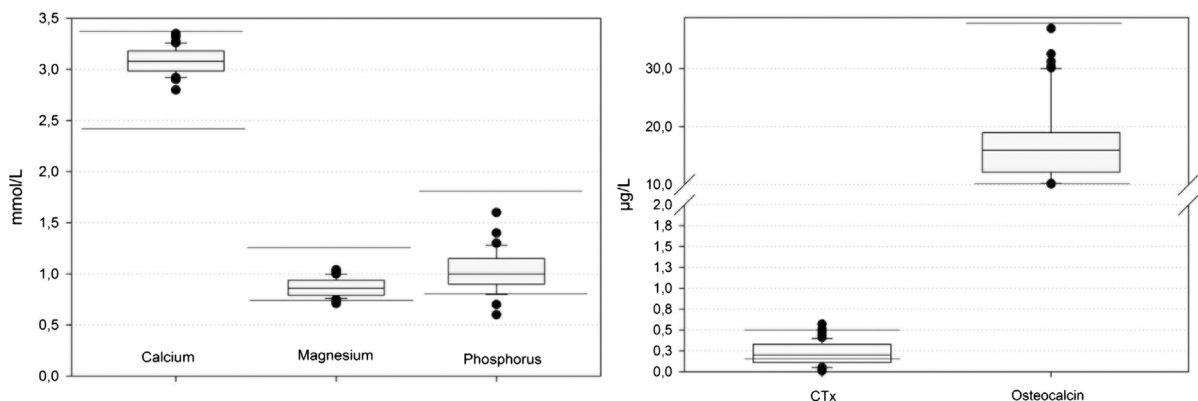


Fig. 1. Boxplot of calcium, magnesium, phosphorus, C-telopeptide type 1, and osteocalcin in blood from mares 2 weeks before calculated parturition (reference values as black line) (study 1).

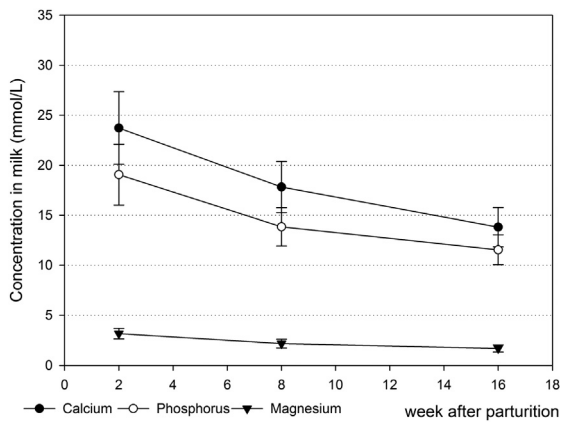


Fig. 2. Concentration of calcium, phosphorus, and magnesium in milk until 16 weeks after parturition (study 1).

severe). Categories A and B were considered as no OC whereas scores C–E were classified as OC.

Statistical analyses of individual results were done with Stata 11 (StataCorp LP, College Station, TX). Graphical analyses were done with SigmaPlot (SigmaPlot for Windows, version 11; Systat Software, Chicago, IL 60606, US). The effect of management parameters was calculated with linear regression and the variables were treated as categorical parameters. The effect of supplementation of magnesium (yes or no) on OC (yes or no) was analyzed with logistic regression and calculating odds ratio. Also the effect of supplementation on the three joints was done with the same model and calculating odds ratios. The model was corrected for observations of the same foal.

3. Results

Blood analysis results of calcium, magnesium, phosphorus, C-telopeptide type 1, and osteocalcin at 2 weeks before parturition are shown in Fig. 1. These values are all within the reference ranges of the laboratory of Animal Health Services.

Average concentrations of calcium, phosphorus, and magnesium in milk are shown in Fig. 2.

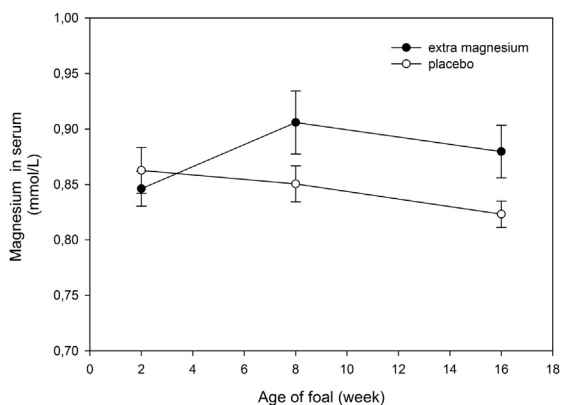


Fig. 3. Magnesium in blood from foals receiving placebo or supplement with magnesium (study 1).

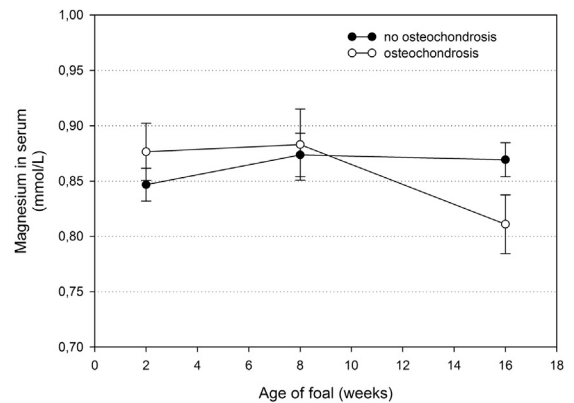


Fig. 4. Magnesium in blood from foals with or without osteochondrosis (study 1).

Fig. 3 shows different serum magnesium concentrations in blood from supplemented and nonsupplemented foals during the age of first 16 weeks. Supplementing magnesium did not have an effect on the concentrations of biomarkers, osteocalcin or CTx-1 (results not shown).

Fig. 4 shows statistically higher magnesium levels ($P < .05$) at week 16 in blood from foals that were scored without OC at an age of 5 months.

The average prevalence of OC in each stud farm is shown in Table 1.

Using the ratio between osteocalcin and CTx-1 as a marker for active bone metabolism, Table 2 shows a significant difference ($P < .05$) in OC prevalence in foals at an age of 8 weeks, based on their bone metabolism. This difference did not exist, however, when foals were aged 5 months (results not shown).

Animals with OC at an age of 5 months had on average, both a lower osteocalcin and a lower CTx-1, at an age of 2–8 weeks. However, this difference was not statistically significant at an age of 16 weeks.

Foals, with no OC detected at an age of 5 months, had a ($P < .01$) higher average magnesium serum level at an age of 16 weeks. There was also a tendency that animals without OC had on average a higher phosphorus serum level; however, this was not statistically significant.

Most management parameters like water holders, drinking system, and feeding system did not differ between stud farms, and therefore, these parameters were not used in further calculations.

Statistical evaluation of the effect of feeding supplement on OC of the three joints in study 1 gave the following result (OC_FP means OC of the FP joint):

OC_FP: odds ratio: 0.8 (95% confidence interval [CI]: 0.4–1.7) ($P = .62$)

OC_MCP/MTP: odds ratio: 2.8 (95% CI: 1.4–5.8) ($P = .004$)

OC_TC: odds ratio: 4.2 (95% CI: 1.5–11.6) ($P = .006$)

Likelihood-ratio test of rho = 0; Chibar = 4 ($P = .02$).

This means that the knee joint was not influenced by magnesium at this age, but fetlock and hock were improved by feeding magnesium.

Table 1
Prevalence of osteochondrosis in Mg-supplemented foals and nonsupplemented foals at an age of 5 months in five stud farms (study 1).

Stud farm	Mg supplementation			
	No		Yes	
	Foals (n)	OC prevalence (%)	Foals (n)	OC prevalence (%)
1	5	20.0	5	40.0
2	12	33.3	13	15.4
3	6	50.0	6	33.3
4	4	75.0	5	20.0
5	4	50.0	3	0.0
Total	31	41.9	32	21.9

Mg, magnesium; OC, osteochondrosis.

In Fig. 5A, the average relative movement (as category varying from 0 = lowest to 3 = highest possibility of exercise) plotted against average OC prevalence per stud farm, shows an almost linear line. One stud farm, feeding foals extra minerals and extra magnesium above the level in the supplement, was clearly deviating. Combining extra feed and movement of the foals, Fig. 5B shows a linear line. Note that the scale is a categorical scale and not a numerical value.

Fig. 6 shows a relation between growth and CTx-1 at an age of 12 months ($P < .001$). No such relation was found between growth and osteocalcin activity (results not shown).

Fig. 7 shows the results of the blood samples for calcium, magnesium, phosphorus, osteocalcin, CTx-1, and bone activity expressed as the ratio between osteocalcin and CTx-1. In Fig. 7F, bone activity of a full-grown mare is pictured with a line (about 80 units).

In the second study, OC prevalence was measured at an age of 5 and 12 months. The change in OC prevalence for supplemented and nonsupplemented foals is listed in Table 3.

At the end of the study, the OC prevalence of the placebo was 42.9% (statistically no change), but the group receiving the supplement had a change in OC prevalence from 56% to 41.7%, which was a statistically significant change. When looking more in detail, it was the OC of the knee joint that improved most. As can be seen from Fig. 7F, some foals have reached the bone activity of a full-grown horse at an age of 12 months.

Fig. 8 shows that supplementation with magnesium resulted in a reduced prevalence of FP (knee joint) OC at an age of 12 months.

Table 2
Bone metabolism (ratio of osteocalcin and CTx-1) of foals aged 8 weeks and osteochondrosis prevalence (study 1).

Stud farm	Bone metabolism at 8 wk			
	Below average		Above average	
	Foals (n)	OC prevalence (%)	Foals (n)	OC prevalence (%)
1	8	12.5	2	100.0
2	15	20.0	10	30.0
3	8	37.5	4	50.0
4	7	28.5	2	100
5	4	25.0	3	33.3
Total	42	23.8	21	47.6

CTx-1, C-terminal telopeptide of type 1 collagen; OC, osteochondrosis.

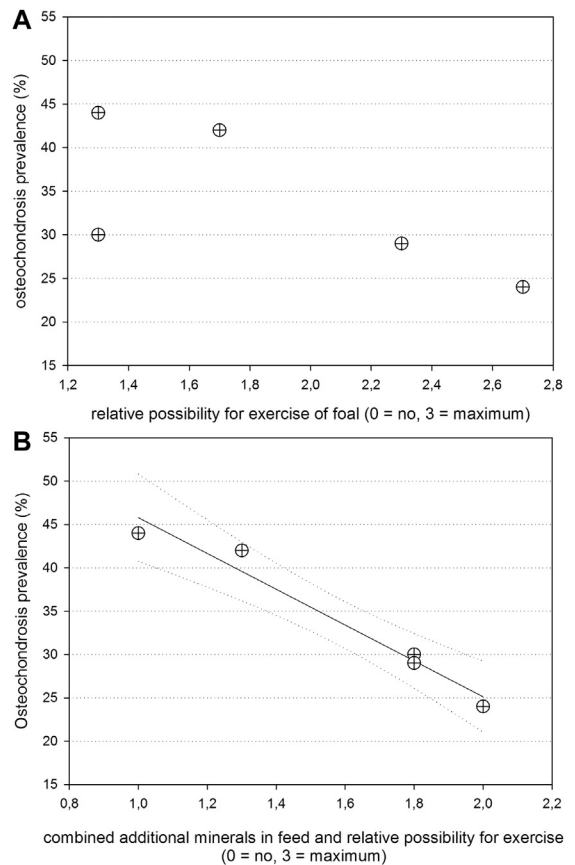


Fig. 5. (A and B) Average prevalence of osteochondrosis per stud farm as a function of relative movement (exercise), alone or in combination with the foal additional feeding minerals (study 1).

Fig. 9 shows OC prevalences of both studies. After supplementing young foals with magnesium at an age of 5 months, the OC prevalence was significantly lower. In the second study, the addition of magnesium lowers the OC prevalence at 12 months compared with the situation at 5 months.

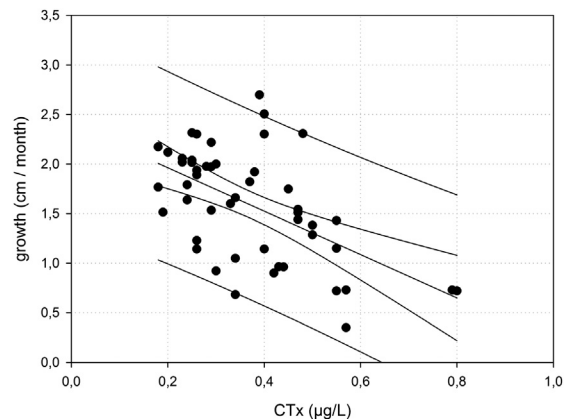


Fig. 6. Relation between growth from 5 to 12 months and the CTx-1 at 12 months (study 2). CTx-1, C-terminal telopeptide of type 1 collagen.

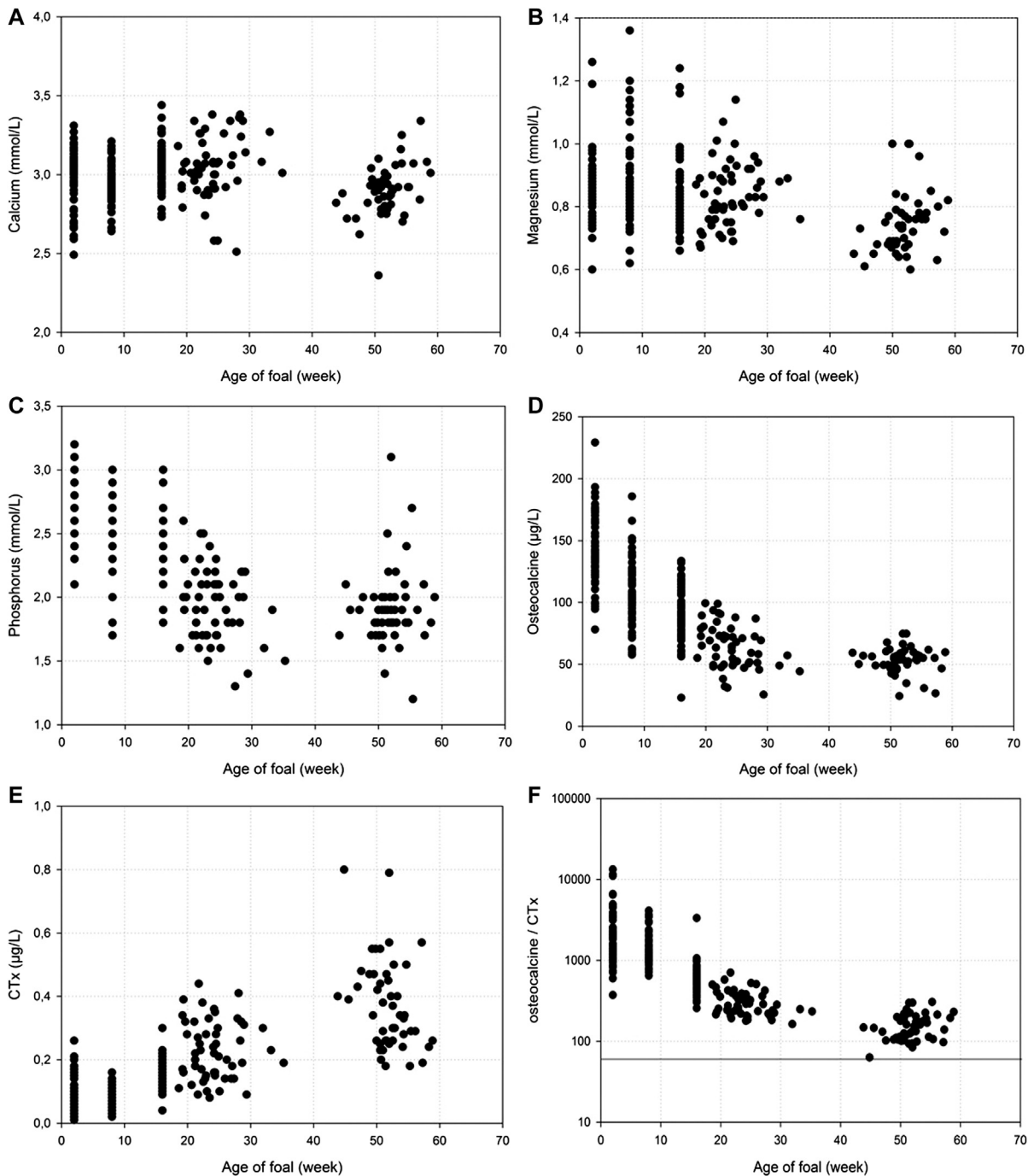


Fig. 7. (A–F) Blood parameters of foals from 2 to 60 weeks (combined study 1 and study 2).

4. Discussion

The two objectives in this study were: to measure the effect of supplementing magnesium in combination with phosphorus during the first 12 months of a foal on the prevalence of OC and to evaluate the use of bone specific biomarkers osteocalcin and CTx-1 as a risk of OC. The factors that have been related to OC are imbalanced feeding, fast growth, exercise, and genetic factors [2,6,12,18].

Especially, the role of copper has been studied [19–21], but also the negative role of zinc and cadmium [22,23]. Housing system also can influence OC in foals [2]. Also in our study, we noted for each stud farm parameters such as physical exercising of the foal. In study 1, foals were housed together with their mares. Depending on the farm, they had the possibility to run outside for several hours (or not). The more space the foal and mare had or the possibility for running outside the less was the OC incidence on that farm.

Table 3

Prevalence of osteochondrosis in Mg-supplemented foals and nonsupplemented foals at an age of 5 and 12 months in six stud farms (study 2).

Stud farm	Mg supplementation					
	No			Yes		
	Foals (n)	OC prevalence (%)		Foals (n)	OC prevalence (%)	
		5 months	12 months		5 months	12 months
1	6	50	33.3	6	33.3	33.3
2	2	50	50	2	100	50
3	7	37.5	28.6	9	70	44.4
4	7	57.1	28.6	1	0	0
5	3	33.3	66.7	3	66.7	33.3
6	3	0	33.3	3	33.3	66.7
Total	28	41.1	42.9	24	56.0	41.7

Mg, magnesium; OC, osteochondrosis.

Although only five stud farms with 63 animals participated in this study, the relation between more space and time spend outside and a lower OC incidence is very suggestive and comparable with other findings [24,25].

The blood minerals and biomarkers of the mares 2 weeks before parturition did not have any influence on the milk composition after parturition (results not shown). The mineral content found in this study is comparable with previous studies [26,27].

The concentrations of osteocalcin and CTx-1 are comparable with other publications [9–11]. The activity of the bone metabolism (expressed as the ratio between osteocalcin and CTx-1) was statistically higher at an age of 8 weeks in foals diagnosed with OC after 5 months compared with animals without OC (23.8% OC in the group with lower bone metabolism compared with 47.7% OC in the group with higher bone metabolism).

In the second study, there was a negative correlation between CTx-1 and the growth of the foal measured as difference in wither height at an age of 12 and 5 months. Previous researchers [13] showed a positive correlation between growth and osteocalcin and a negative correlation between CTx-1 and growth. In this second study, there was

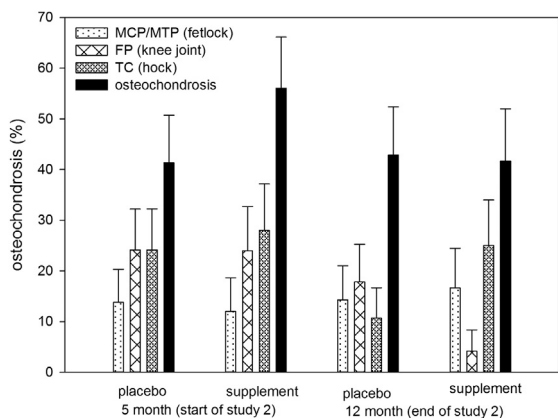


Fig. 8. Prevalence of osteochondrosis of three joints (MCP and/or MTP, FP, and TC) at the age of 5 and 12 month, with and without feeding of additional magnesium (study 2). FP, femoropatellar; MCP, metacarpophalangeal; MTP, metatarsophalangeal; TC, tarsocrural.

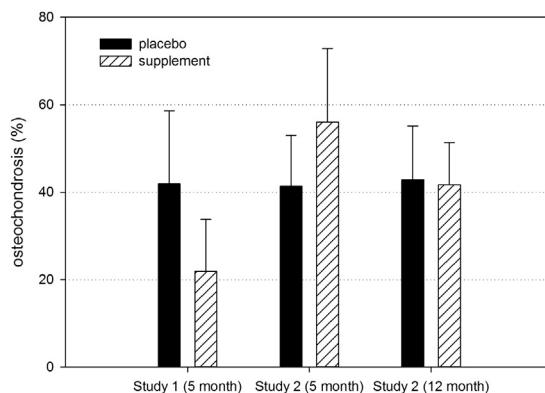


Fig. 9. Prevalence of osteochondrosis in study 1 and study 2 in supplemented and nonsupplemented foals.

not a clear relation between the bone metabolism as measured by osteocalcin and CTx-1. Therefore, we concluded that osteocalcin and CTx-1 as biomarkers for bone metabolism cannot be used as indicators for risk of OC.

As expected, feeding additional magnesium to foals resulted in higher magnesium in blood. In the first study, foals without OC had significant lower magnesium in their blood at an age of 16 weeks. The average prevalence of OC in this first study was statistically different in the placebo group (OC prevalence = 41.9%) compared with the group receiving the magnesium supplement (OC prevalence = 21.9%). The second study started with foals at an average age of 5.5 months. The average OC prevalence was 48.7%. Foals were randomly assigned in the supplement or placebo group. The OC score was measured without knowledge about the group the foal was placed to the researchers doing the interpretation of the X-ray images. At the end of the second study, again the foals were radiographed but again blind. Only when all results were combined, it revealed that the placebo group had an average OC prevalence of 41.4% and the supplement group 56.0%. At the end of the study, the placebo group had still a prevalence of 42.9% whereas the supplement group ended with 41.7%, a decrease of 14.3% prevalence. It is well-known that several joints can heal on a natural way during the first 12 months of a foal [6]. As the foals were randomly divided into two groups, this would be the same in both groups. Therefore, the differences in OC prevalence at the end of study 2 are not likely to be an effect of natural healing, but supplementation.

The conclusion of our both studies is that magnesium supplementation and more exercise of the foal could lower the OC prevalence significantly. Mainly the OC prevalence of the knee joint was very low after supplementation of magnesium.

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Author contributions Guillaume Counotte (Animal Health Services): study setup, supervision of laboratory testing, statistical analysis, and preparing the manuscript. Gerrit Kampman (Den Ham): sampling the foals, taking the X-rays, and interpretation of the X-rays. Vincent Hinnen calculated uptake of minerals for each foal and stud farm and made the placebo and supplement pastes.

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