

# Prepartum measurement of serum biomarkers reflecting osteoclastic and osteoblastic bone metabolism for predicting the risk of milk fever in dairy cows

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## SUPPLEMENTARY FILE

**Supplementary Table S1.** Ingredient and nutritional composition of the prepartum and postpartum rations during the study involving 58 Holstein cattle

Items	Prepartum ration <sup>1</sup>	Postpartum ration <sup>2</sup>
Ingredient (%DM)		
Grass silage	58.7	22.1
Corn silage	13.5	37.8
Grain <sup>3</sup>	0.2	1.9
Soy bean meal	0	3.8
Concentrate mix <sup>4</sup>	34.1	34.4
Vitamin and mineral <sup>5</sup>	2.0	0.1
Dry Matter (%)	31.5	39.3
Nutrition composition		
Crude Protein (%DM)	13.0	16.7
Ca (%DM)	0.86	0.53
P (%DM)	0.39	0.46
K (%DM)	1.85	1.70
Mg (%DM)	0.16	0.26
S (%DM)	0.48	0.25
Na (%DM) <sup>6</sup>	0.10	0.11
Cl (%DM)	0.44	0.49
DCAD (mEq/100g DM)	9.29	18.8

<sup>1</sup> From 28 d to 1 d prepartum.

<sup>2</sup> After calving to mid-lactation.

<sup>3</sup> Contained beat pulp, ground corn and rice bran

<sup>4</sup> Nutrient composition on a DM basis: 18% CP, 2.0% Crude Fat, 10% Crude Fiber, 0.50% Ca and 0.4% P; 74.0% TDN, including corn, corn gluten feed, corn distillers byproducts, grain soluble, bran, rapeseed and soy-bean cake; Doto Siryo Co., Ltd, Japan)

<sup>5</sup> Including vitamin E, Mepron, Calcium Carbonate, Calcium Sulfer and Vitamin mix

<sup>6</sup> A lack of Na intake was compensated by mineral salt block

## Supplementary Table S2:

**Supplementary Table S2.** Actual sampling days before calving (mean and standard error of mean) of prepartum samples analyzed in each group or MF and non-MF cows

Groups/Cows	n	-3 wk	-2 wk	-1 wk
NP group	13	-20.6 ± 0.5	-13.7 ± 0.5	-6.5 ± 0.4
PP group	20	-20.5 ± 0.5	-13.3 ± 0.5	-6.6 ± 0.4
M2 group	13	-20.8 ± 0.4	-13.7 ± 0.4	-6.4 ± 0.4
M3 group	12	-20.6 ± 0.5	-13.5 ± 0.5	-6.5 ± 0.5
non-MF cows	17	-20.8 ± 0.4	-13.8 ± 0.4	-6.5 ± 0.4
MF cows	8	-21.0 ± 0.6	-12.9 ± 0.4	-5.8 ± 0.5

NP, nulliparous; PP, primiparous; M2, multiparous in 2nd lactation; M3, multiparous in 3rd–5th lactation; MF, milk fever.

There were no statistical difference in days at each timepoint among the groups (one way ANOVA) or between the cows (Student's *t*-test).