Effects of oral supplementation with organically modified clinoptilolite during prepartum period on colostrum quality in primiparous dairy cows

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Supplementary File

Supplementary Methods

Analysis of colostrum

Colostrum samples were analyzed for total dry matter, protein and fat content (%), and pH value. Gravimetric method (drying at 102°C) for dry matter content determination was used (ISO 6731:2010). Total protein content (%) was analyzed according to the Kjeldahl method (ISO 8968-1:2014). Samples of colostrum were digested (Gerhardt, Kjeldatherm KB/KBL, Germany) and distilated (Gerhardt, Vapodest 20, Germany). Conversion factor (6.38) was used to convert the measured nitrogen concentration to a protein concentration. Fat content (%) was analyzed according to the butyrometric methods (ISO 11870:2009). The fat in colostrum samples were extracted by centrifugation on 1100 rpm using Gerber centrifuge (CEVO 65, Berlin, Germany). Fat content was read directly on the scale of the butyrometer at 65°C. pH value was determined by potentiometric measurements using pH meter (Extech A922809) according to AS2300.1.6-210.

Table S1. Ingredients and chemical composition of the close up and lactation diets

Ingredient, % of DM	Diet	
	Close up	Lactation
Corn silage	47.51	39.8
Alfalfa hay	9.35	6.59
Wheat straw	2.39	1.00
Alfalfa silage	9.35	13.24
Ground shelled corn	4.67	6.84
Triticale silage	4.22	5.12
Brewer grain		4.31
Barley	3.98	4.23
Dried beet pulp	8.59	9.11
Sunflower meal	4.67	
Soybean meal	2.39	4.13
Soybean grits	0.93	4.13
Minerals	1.95	1.5
Analyzed nutrient composition		
	Close up	Lactation
Crude protein, % of DM	14.88	16.12
Fat, % of DM	2.80	2.1
NEL, Mcal/kg	1.60	1.71
ADF, % of DM	24.12	21.39
NDF, % of DM	37.84	36.72

Supplementary Results

Table S2. Colostrum quality parameters of control (C0) and organically modified clinoptilolite treated group (C150) of primiparous dairy cows

		Colostrum											
		1st (2-3h)			2nd (12h)			3rd (24h)			4th (36h)		
		C0	C150	↑↓	C0	C150	↑↓	C0	C150	↑↓	C0	C150	↑↓
Dry matter	x̄ ± SD	28.7 ± 3.2	30.2 ± 4.4	/	18.5 ± 3.4	24.2 ± 6.4	30 % ↑	15.2 ± 2.6	16.8 ± 3.4	/	14.5 ± 1.3	14.9 ± 1.8	/
(%)	SE	0.8	1.0		0.8	1.4		0.7	0.8		0.3	0.4	
	Iv	21.1-33.7	23.3-40.1		14.5-26.6	14.3-36.0		11.6-20.1	12.7-23.6		12.7-16.8	12.1-20.2	
	Cv %	11	14		18	26		17	20		9	12	
Total protein	x̄ ± SD	13.5 ± 2.3	15.6 ± 1.9	15 % ↑	8.4 ± 2.9	10.9 ± 2.9	30 % ↑	5.7 ± 1.3	6.3± 1.6	/	4.7 ± 1.0	5.0 ± 0.8	/
(%)	SE	0.6	0.4		0.7	0.7		0.3	0.3		0.3	0.2	
	Iv	9.0-17.8	12.6-19.7		5.1-15.2	6.0-16.6		3.7-7.9	4.4-9.6		3.0-7.1	3.8-6.9	
	C_V %	17	12		34	27		23	25		21	17	
Fat	x̄ ± SD	8.5 ± 2.4	7.7 ± 2.2	/	5.3 ± 1.6	7.7 ± 2.6	45 % ↑	4.5 ± 1.4	5.6 ± 1.7	/	4.7 ± 1.2	5.2 ± 1.0	/
(%)	SE	0.6	0.5		0.4	0.6		0.3	0.4		0.3	0.2	
	Iv	4.4-12.1	2.6-11.0		2.2-8.4	4.2-14.5		2.6-7.0	3.5-9.0		2.9-7.7	4.0-7.0	
	C_V %	28	28		30	33		30	30		26	18	
pH	x̄ ± SD	6.23±0.08	6.17 ±0.09	/	6.29 ±0.08	6.25 ±0.09	/	6.30 ±0.11	6.28 ±0.13	/	6.36 ±0.13	6.30 ±0.11	/
	SE	0.02	0.02		0.02	0.02		0.03	0.03		0.03	0.02	
	Iv	6.15-6.45	6.03-6.40		6.16-6.44	6.08-6.47		6.15-6.61	6.10-6.52		6.25-6.77	6.11-6.47	
	C_V %	1	1		1	1		2	2		2	2	

Table S2. *Continued*

		Colostrum											
		1st (2-3h)			2nd (12h)			3rd (24h)			4th (36h)		
		C0	C150	↑↓	C0	C150	↑↓	C0	C150	↑↓	C0	C150	↑↓
%Brix	x̄ ± SD	25.5 ± 3.4	28.2 ± 3.5	11 % ↑	16.4 ± 3.3	21.2 ± 5.3	29 % ↑	12.7 ± 1.7	14.2 ± 2.5	/	12.0 ± 0.9	12.3 ± 1.2	/
	SE	0.9	0.8		0.8	1.2		0.4	0.6		0.2	0.3	
	Iv	18.0-30.8	23.7-35.7		11.9-23.5	15.3-31.3		10.4-16.0	11.4-19.3		10.4-14.1	11.0-15.3	
	C_V %	13	13		20	25		13	18		7	10	
Volume (L)	x̄ ± SD	4 ± 2	5 ± 3	/	3 ± 2	4 ± 2	/	5 ± 2	6 ± 2	/	6 ± 2	6 ± 2	/
	SE	1	1		1	1		1	1		1	1	
	Iv	1-6	1-12		1-7	1-8		1-8	3-12		3-10	4-10	
	C_V %	41	64		50	43		44	42		34	26	
IgG concentration (g/L)	x̄ ± SD	130 ± 33	157 ± 34	21 % ↑	57 ± 30	88 ± 40	54 % ↑	21 ± 15	35 ± 24	/	10 ± 7	12 ± 9	/
	SE	8	8		7	9		4	5		2	2	
	Iv	65-165	104-240		15-131	36-152		2-66	12-81		2-27	2-36	
	C_V %	26	22		52	45		73	69		72	79	
IgG mass (g)	x̄± SD	481 ± 264	662 ± 396	38% ↑	184 ± 109	301 ± 156	/	125 ± 90	167 ± 116	/	64 ± 45	72± 47	/
	SE	70	96		29	38		24	28		12	12	
	Iv	135-990	208-1572		25-357	72-600		48-396	78-486		12-162	16-180	
	C_V %	55	60		59	52		72	70		71	66	

C: control group of cows; T: cows orally supplemented with organically modified clinoptilolite (treated group); (↑↓): percentage of increase (↑) or decrease (↓) in treated compared to control group; (/) no difference between the groups; x̄ ± SD: mean ± standard deviation; SE: standard error of mean; Iv: minimum to maximum range; Cv: coefficient of variation.

Table S3. The significance of difference in values of parameters defining colostrum quality of control (C; i.e. C0 - see in the Material and methods) and clinoptilolite treated (T; i.e. C150 - see in the Material and methods) primiparous dairy cows

Colostrum: Solids (%)								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	<0.0001	<0.001	<0.0001	ns			
C 12h		/	0.004	0.0002		<0.0001		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	<0.0001	<0.0001	<0.0001
T 12h						/	<0.0001	<0.0001
T 24h							/	ns
T 36h								/
Colostrum: %Brix								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	<0.0001	<0.0001	<0.0001	0.046			
C 12h		/	<0.0001	<0.0001		<0.0001		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	<0.0001	<0.0001	<0.0001
T 12h						/	<0.0001	<0.0001
T 24h							/	ns
T 36h								/
Colostrum: Fat (%)								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	<0.0001	<0.0001	<0.0001	ns			
C 12h		/	ns	ns		0.0005		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	ns	0.0004	<0.0001
T 12h						/	0.0003	<0.0001
T 24h							/	ns
T 36h								/
Colostrum: Volume (L)								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	ns	ns	0.0009	ns			
C 12h		/	ns	0.0002		ns		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	ns	ns	ns
T 12h						/	ns	0.01
T 24h							/	ns
T 36h								/

Table S3. *Continued*

Colostrum: Protein (%)								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	<0.0001	<0.0001	<0.0001	0.008			
C 12h		/	<0.0001	<0.0001		0.0009		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	<0.0001	<0.0001	<0.0001
T 12h						/	<0.0001	<0.0001
T 24h							/	0.03
T 36h								/
Colostrum: pH								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	ns	0.0111	<0.0001	ns			
C 12h		/	ns	0.009		ns		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	0.0003	<0.0001	<0.0001
T 12h						/	ns	ns
T 24h							/	ns
T 36h								/
Colostrum: IgG (g/L)								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	<0.0001	<0.0001	<0.0001	0.02			
C 12h		/	<0.0001	<0.0001		0.004		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	<0.0001	<0.0001	<0.0001
T 12h						/	<0.0001	<0.0001
T 24h							/	0.001
T 36h								/
Colostrum: IgG (g)								
P	C 2h	C 12h	C 24h	C 36h	T 2h	T 12h	T 24h	T 36h
C 2h	/	0.0009	<0.0001	<0.0001	0.04			
C 12h		/	ns	ns		ns		
C 24h			/	ns			ns	
C 36h				/				ns
T 2h					/	<0.0001	<0.0001	<0.0001
T 12h						/	ns	0.007
T 24h							/	ns
T 36h								/

Table S4. Concentration of γ globulin and relative content of cationic and anionic γ globulin fractions in peripheral blood serum of control (C0) and organically modified clinoptilolite treated group (C150) of primiparous dairy cows

		Blood serum														
Day		-24 ± 5			-4 ± 2			+1			+2			+7		
		C0	C150	↑↓	C0	C150	↑↓	C0	C150	↑↓	C0	C150	↑↓	C0	C150	↑↓
γ globulin‡	x̄ ± SD	38 ± 5	39 ± 5	/	39 ± 6	37 ± 5	/	37 ± 7	40 ± 6	/	38 ± 3	38 ± 5	/	44 ± 6	45 ± 6	/
(g/l)	SE	2	1		1	5		2	2		1	1		2	1	
	Iv	30-47	31-48		30-56	24-49		28-57	29-48		33-45	31-49		34-56	36-55	
	Cv %	14	13		17	13		20	16		9	13		15	13	
Cationic	x̄ ± SD	57 ± 3	59 ± 5	/	70 ± 5	65 ± 3	7% ↓	61 ± 6	65 ± 3	7% ↑	66 ± 4	64 ± 5	/	63 ± 5	63 ± 4	/
γ globulin	SE	1	1		1	1		2	1		1	1		1	1	
(%)#	Iv	52-63	50-66		63-80	60-70		50-70	61-74		60-72	56-70		50-69	58-73	
	Cv %	6	8		7	4		9	5		5	7		8	6	
Anionic	x̄ ± SD	43 ± 3	41 ± 5	/	30 ± 5	35 ± 3	17% ↑	39 ± 6	35 ± 3	10 % ↓	34 ± 4	36 ± 4	/	37 ± 5	37 ± 4	/
γ globulin	SE	1	1		1	1		2	1		1	1		1	1	
(%)#	Iv	37-48	34-50		20-37	30-40		30-50	26-39		28-40	30-44		31-50	27-42	
	Cv %	8	12		16	7		15	9		10	13		14	11	

Day: day before (-) or after calving (+); C: control group of cows; T: cows orally supplemented with organically modified clinoptilolite (treated group); (‡) concentration of γ globulin calculated based on relative concentration (%) of γ globulin (obtained by densitometry of serum protein electrophoretogram) and the concentration of total serum protein determined by Biuret test. (#) percentage of total γ globulin; (↑↓): percentage of increase (↑) or decrease (↓) in treated compared to control group; (/) no difference between the groups; x̄ ± SD: mean ± standard deviation; SE: standard error of mean; Iv: minimum to maximum range; Cv: coefficient of variation.

Table S5. The significance of difference in the concentration of total γ globulin and γ globulin fractions in peripheral blood serum of control (C; i.e. C0 - see in the Material and methods) and clinoptilolite treated (T; i.e. C150 - see in the Material and methods) primiparous dairy cows

d - day of experiment; (-) – prepartum; (+) – postpartum.

γ globulin (g/L)										
Control group overall (last square) means: 39 g/L; Treated group overall (last square) means: 40 g/L; SEM = 1.448 $P_{(T)}$ 0.4433; $P_{(TxD)}$ 0.4340										
P	C d-21	C d-5	C d+1	C d+2	C d+7	T d-21	T d-5	T d1	T d+2	T d+7
C d-21	/	ns	ns	ns	0.01	ns				
C d-5		/	ns	ns	0.03		ns			
C d+1			/	ns	0.0006			ns		
C d+2				/	0.008				ns	
C d+7					/					ns
T d-21						/	ns	ns	ns	0.001
T d-5							/	ns	ns	<0.0001
T d+1								/	ns	0.004
T d+2									/	<0.0001
T d+7										/
"Slow" cationic γ globulin (% of total γ globulin)										
Control group overall (last square) means: 63%; Treated group overall (last square) means: 63%; SEM = 0.885 $P_{(T)}$ 0.8930; $P_{(TxD)}$ 0.0005.										
P	C d-21	C d-5	C d1	C d2	C d7	T d-21	T d-5	T d1	T d2	T d7
C d-21	/	<0.0001	ns	<0.0001	0.002	ns				
C d-5		/	<0.0001	ns	0.0005		0.02			
C d+1			/	0.02	ns			0.02		
C d+2				/	ns				ns	
C d+7					/					ns
T d-21						/	<0.0001	<0.0001	0.0013	0.0048
T d-5							/	ns	ns	ns
T d+1								/	ns	ns
T d+2									/	ns
T d+7										/
"Fast" anionic γ globulin (% of total γ globulin)										
Control group overall (last square) means: 37%; Treated group overall (last square) means: 37%; SEM = 0.875 $P_{(T)}$ 0.8988; $P_{(TxD)}$ 0.0005.										
P	C d-21	C d-5	C d1	C d2	C d7	T d-21	T d-5	T d1	T d2	T d7
C d-21	/	<0.0001	ns	<0.0001	0.002	ns				
C d-5		/	<0.0001	ns	0.0005		0.02			
C d+1			/	0.02	ns			0.02		
C d+2				/	ns				ns	
C d+7					/					ns
T d-21						/	<0.0001	<0.0001	0.001	0.005
T d-5							/	ns	ns	ns
T d+1								/	ns	ns
T d+2									/	ns
T d+7										/