

**Comparison of marine algae-based rumen buffer with sodium bicarbonate buffer and its role in increasing milk value**

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**SUPPLEMENTARY FILE**

**31 Supplementary Table S1.** Ingredients and chemical composition of basal TMR and concentrate of the experimental diets

Item	Treatments <sup>1</sup>	
	CON	LITHO
Inclusion proportion	g/kg/DM	
Roughage	460	460
Concentrate	540	540
Ingredient of the TMR	g/kg/DM	
Water	0.1	0.1
Corn Silage	318.6	318.6
Pre-dried ryegrass	438.6	438.6
Concentrate	896.7	896.7
Sodium bicarbonate	280	-
<i>Lithothamnium calcareum</i>	-	102
Ingredient of the Concentrate	g/kg/DM	
Wholegrain rice bran	126.5	126.5
Milled grain corn	387.5	387.5
Soy hull	169.8	169.8
Soybean meal	228.3	228.3
Bypass protein	50.9	50.9
Livestock Urea	8.2	8.2
Protected Fat	14.0	14.0
Mineral Premix	3.9	3.9
Common salt	10.9	10.9
TMR chemical analysis		
DM (g/kg/DM)	464.7	464.7
Mineral Matter (g/kg/DM)	72.6	72.6
Crude Protein (g/kg/DM)	167.6	167.6
Ether extract (g/kg/DM)	45.6	45.6
NDF (g/kg/DM)	326.2	326.2
ADF (g/kg/DM)	198.7	198.7
Starch (g/kg/DM)	292.8	292.8
NFC (g/kg/DM)	388.0	388.0
DCAD meq/100gr	283.7	283.7

**32** DM = dry matter. TMR = totally mixed ration. NDF = neutral detergent fibre. ADF = acid detergent fibre. NFC = Non fibrous carbohydrates.

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**Supplementary Table S2.** Feeding behavior and activity of Holstein cows fed diets supplemented with sodium bicarbonate (CON) or *Lithothamnium calcareum* (LITHO)

Parameters	LSMEANS±SE		Treatment	P-value	
	CON	LITHO		Period	TxP
Freq <sup>3</sup> . access troughs/day	66.88±4.10	69.20±3.98	0.65	<0.01	0.02
Number of meals (N <sup>o</sup> /day)	33.18±2.14	33.85±2.07	0.78	<0.01	0.01
Meal size (kg/meal)	1.15±0.05	1.14±0.05	0.99	<0.01	0.01
Eating duration (min/meal)	5.77±0.35	5.75±0.35	0.95	<0.01	<0.01
Rumination (min/day)	593.93±4.81	601.67±5.02	0.27	<0.01	0.15
Activity (min/day)	230.19±8.89	215.29±9.15	0.25	<0.01	0.70
Idle (min/day)	605.28±9.79	615.82±9.48	0.44	<0.01	0.78

<sup>ab</sup>Within a line, different superscript lowercase letters denote significant differences (P<0.05) among the samples.

LSMEANS ± standard error of the mean (SE).

TxP = Treatment interaction with period.

Freq = frequency.

**Supplementary Table S3.** Blood metabolite and mineral concentrations of Holstein cows fed diets supplemented with sodium bicarbonate (CON) or *Lithothamnium calcareum* (LITHO)

Parameters	LSMEANS±SE		Treatment	P-value	
	CON	LITHO		Period	TxP
Total Protein (g/dL)	8.09 ± 0.09	7.87 ± 0.09	0.10	0.05	0.43
Albumin (g/dL)	2.77 ± 0.03	2.73 ± 0.03	0.20	0.09	0.60
Urea (mg/dL)	28.39± 1.60	28.80 ± 1.59	0.84	<0.01	0.12
Glucose (mg/dL)	59.96 ± 0.76	61.09 ± 0.76	0.29	<0.01	0.44
Calcium (mg/dL)	8.58 ± 0.10	8.52 ± 0.10	0.57	0.19	0.99
Magnesium (mg/dL)	2.24 ± 0.05	2.26 ± 0.05	0.83	0.75	0.22
NEFA (mmol/L)	0.43 ± 0.03	0.35 ± 0.03	0.07	<0.01	0.76
βHBA (mmol/L)	0.47 ± 0.02	0.48 ± 0.02	0.92	0.86	0.13
PON (U/mL)	129.08±4.58	117.56±4.55	0.09	0.07	0.78

58 TxP = Treatment interaction with period. NEFA = Non-esterified fatty acids. BHBA = Beta-hydroxybutyrate. PON = Paraoxonase.

**Supplementary Table S4.** Blood acid base balance of Holstein cows fed diets supplemented with sodium bicarbonate (CON) or *Lithothamnium calcareum* (LITHO)

Parameters	LSMEANS±SE		Treatment	P-value	
	CON	LITHO		Period	TxP
Blood pH	7.46 ± 0.02	7.45 ± 0.02	0.85	0.23	0.21
HCO <sub>3</sub> <sup>5</sup>	31.91± 1.08	30.66 ± 1.06	0.46	0.61	0.56
pCO <sub>2</sub> <sup>4</sup>	45.02 ± 1.63	44.91 ± 1.64	0.96	0.08	0.87
pO <sub>2</sub> <sup>2</sup>	72.77± 8.03	72.52 ± 7.81	0.98	0.47	0.50

TxP = Treatment interaction with period. HCO<sub>3</sub> = Bicarbonate. pCO<sub>2</sub> = Partial carbon dioxide pressure. pO<sub>2</sub> = Partial oxygen pressure.