

1 **Supplementary Materials**

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3 **Supplementary Table S1.** Ingredients and chemical composition of diets

Items	% of DM
Ingredient	
Alfalfa hay	13.6
Guinea grass	2.0
Corn silage	17.0
Oat grass	5.6
Whole cottonseed	6.3
Beet pulp	5.0
Concentrate supplement	50.5
Chemical analysis, % DM	
CP	16.9
NE _L , Mcal/kg of DM	1.7
NDF	36.7
ADF	22.1
Ca	1.05
P	0.42

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7 **Supplementary Table S2.** Temperature and humidity for the dairy cows used in the study

Item	Non-HS			HS		
	Temp. (°C)	RH (%)	THI	Temp. (°C)	RH (%)	THI
Day 3	06:00	7.7	65.3	48.3	26.0	94.9
	14:00	15.2	54.8	59.2	29.3	93.9
	22:00	9.6	62.5	51.2	28.1	93.9
Day 2	06:00	7.5	67.1	47.9	26.3	94.9
	14:00	14.9	56.2	58.8	28.6	93.9
	22:00	9.2	65.2	50.5	28.1	93.9
Day 1	06:00	7.1	66.4	47.4	26.4	94.9
	14:00	14.1	57.3	58.0	28.7	93.9
	22:00	9.1	64.7	50.4	27.8	93.9

8 Day 3, day2, and day 1 are respective time points before sample collection of dairy cows.

9 RH, relative humidity; Temp., temperature.

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18 **Supplementary Table S3.** Rectal temperature and respiration rate of dairy cows

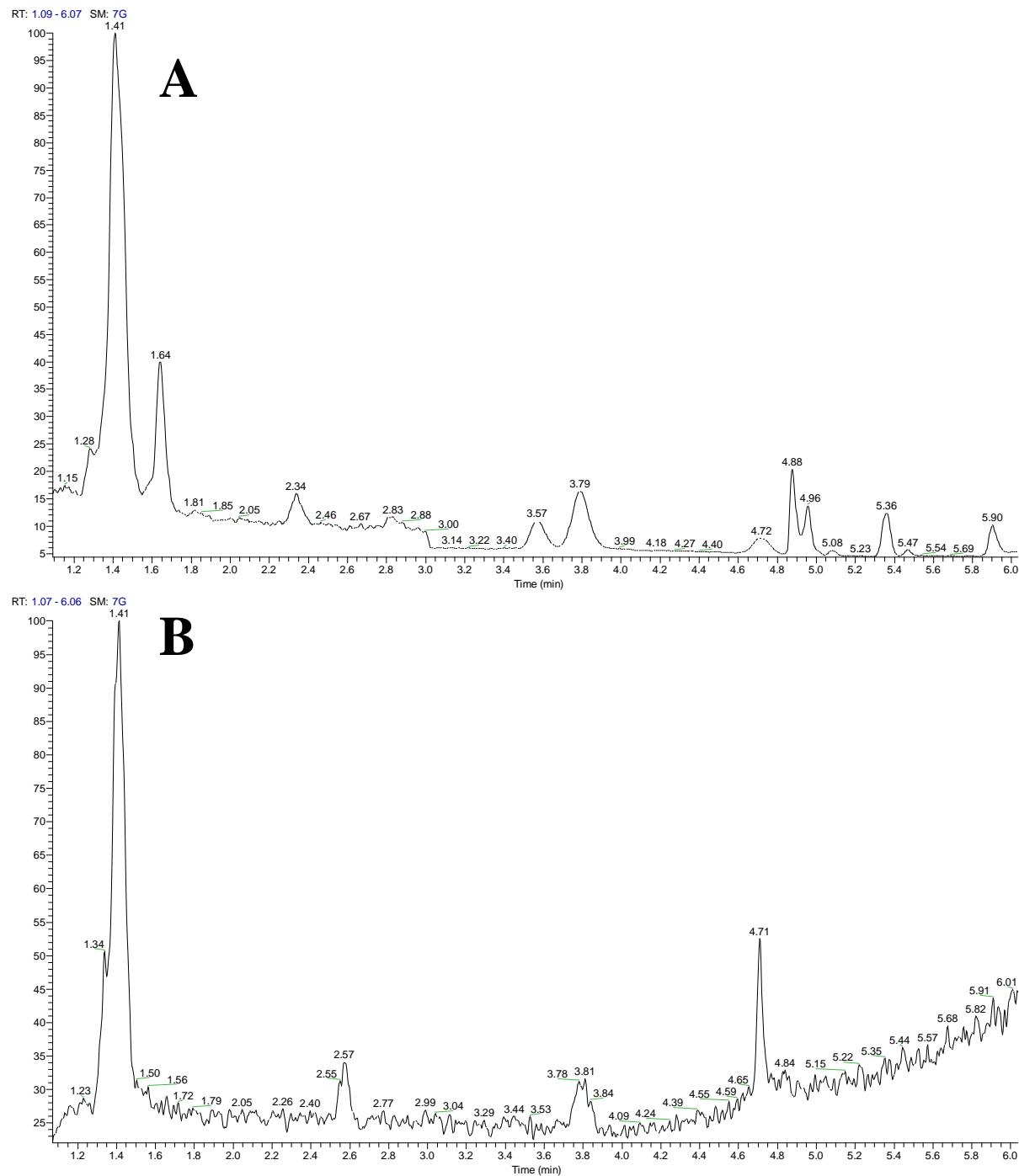
Parameters	Non-HS	HS	P-value
Rectal temperature (°C)			
06:00	38.12 ± 0.07	39.17 ± 0.07	< 0.01
14:00	38.46 ± 0.07	39.53 ± 0.08	< 0.01
22:00	38.31 ± 0.07	39.37 ± 0.08	< 0.01
Respiration rate (breaths/min)			
06:00	42.6 ± 3.3	71.5 ± 4.0	< 0.01
14:00	48.7 ± 3.8	75.8 ± 4.5	< 0.01
22:00	44.2 ± 3.5	72.6 ± 4.3	< 0.01

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20 **Supplementary Table S4.** Production parameters of dairy cows

Parameter	Non-HS	HS	P-value
DMI ¹ , kg/d	24.87 ± 1.76	18.45 ± 1.54	< 0.01
Milk yield, kg/d	37.6 ± 2.4	28.3 ± 2.1	< 0.01
Milk component			
Fat, %	3.48 ± 0.15	3.33 ± 0.12	0.39
Protein, %	3.25 ± 0.11	2.91 ± 0.14	< 0.01
Lactose, %	4.96 ± 0.12	4.88 ± 0.13	0.91
MUN ² (mg/100 mL)	13.76 ± 0.91	11.52 ± 0.73	0.01
SCC ³ (×10 ³ /mL)	112.35 ± 20.23	365.61 ± 43.25	< 0.01

21 ¹DMI, dry matter intake. ²MUN, milk urea nitrogen. ³SCC, somatic cell count.



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23 **Supplementary Fig. S1** Total ion chromatogram acquired by LC-MS-based detection of
24 liver sample in positive ion mode (A) and in negative ion mode (B).