

Contrasting effects of high-starch and high-sugar diets on ruminal function in cattle

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SUPPLEMENTARY FILE

MATERIAL AND METHODS supplementary materials

SAA determination

SAA concentration was determined using a commercially available ELISA kit (SAA-11, Cow serum amyloid A ELISA, Life Diagnostic Inc., UK). The test involved two SAA specific antibodies. SAA is first dissociated from possible interfering factors by heating the sample at 60°C for 1 h and then, once diluted, incubated in antibody coated microtiter wells together with HRP conjugate for 1 h. The wells are then washed to remove unbound antibodies, incubated with chromogen reagents and absorbance is measured at 450 nm.

Hp determination

The Hp determination was performed as described by (Eckersall et al., 1999) with minor modifications. Briefly, 3 µL standards and plasma samples were mixed with 150 µL of diluted haemoglobin (60 mg/L Hb, 0.154 M NaCl). At 50 and 75 sec, 135 µL of chromogen and 37.5 µL of hydrogen peroxide (H₂O₂) were added respectively. Absorbance was measured at 5 min. at a wavelength of 660 nm after H₂O₂ addition.

Histamine determination

The concentration of histamine in ruminal fluid was obtained with a competitive ELISA kit (KA 1888 Histamine ELISA kit, Abnova, Tw). The principle of the test involves the acylation of

histamine and the subsequent incubation of unlabeled antibody in the presence of its antigen (within the specimen). These bound antibody/antigen complexes are then added to an antigen coated well. Unbound antibodies are removed by washing. Absorbance is measured within 10 min at 450 nm.

RESULTS supplementary materials

Table S1 - supplementary materials. Contingency table for the effects of treatment on the binary health indicators and feed consumption. It refers to the section: Feed Intake and Health Parameters Analysis

Variable	Level	Control	Starch	Sugar	<i>P</i> -value
Incomplete feeding	Incomplete	0	5	9	< 0.001
	Complete	30	25	21	
Diarrhoea	Yes	0	9	8	0.005
	No	30	21	22	
Depression	Yes	0	2	0	0.129
	No	30	28	30	
Tympany	Yes	0	0	0	NA
	No	30	30	30	

The *P*-values presented here are the overall *P*-values derived from Chi-sq analysis of contingency tables for each.

Table S2 - supplementary materials. Duration (min/d) below pH thresholds of 5.8 and 5.6, by sequence day and diet. Values expressed as mean (minimum-maximum)

Sequence Day	Control Diet	Starch Diet	Sugar Diet
pH < 5.8			
D-1	8.30 (0.00-40.0)	41.7 (0.00-250)	53.3 (0.00-290)
D-2	0.00 (0.00-0.00)	220 (20.0-460)	323 (0.00-790)
D-3	0.00 (0.00-0.00)	215 (0.00-500)	322 (50.0-610)
D-4	0.00 (0.00-0.00)	152 (0.00-700)	193 (0.00-820)
D-5	41.7 (0.00-250)	78.3 (0.00-170)	167 (0.00-410)
pH < 5.6			
D-1	0.00 (0.00-0.00)	0.00 (0.00-0.00)	26.7 (0.00-160)
D-2	0.00 (0.00-0.00)	51.2 (0.00-190)	165 (0.00-560)
D-3	0.00 (0.00-0.00)	132 (0.00-340)	195 (10.0-530)
D-4	0.00 (0.00-0.00)	107 (0.00-630)	86.7 (0.00-500)
D-5	33.0 (0.00-200)	11.7 (0.00-30.0)	100 (0.00-370)

Table S3 - supplementary materials. Results of simulated sampling of reticuloruminal pH

	Control	High Starch	High Sugar
	(pH)	(pH)	(pH)
	6.19	5.05	5.27
	6.31	5.73	5.74
	6.52	5.61	5.54
	6.36	6.71	6.59
	6.26	6.00	6.10
	6.36	5.97	6.21
	6.07	5.69	5.51
	6.21	5.69	5.89
	6.50	6.31	6.07
	6.50	6.16	6.04
	6.23	5.22	6.28
	6.25	5.62	5.91
Total samples pH < 5.8	0/12	7/12	4/12
Total samples pH < 5.6	0/12	2/12	3/12

Two samples from each of six animals on each of the diets were randomly taken from the pool of all pH values from between 16:00 and 17:00 as described by Jonsson et al. (2019)

Figure S1 - supplementary materials.

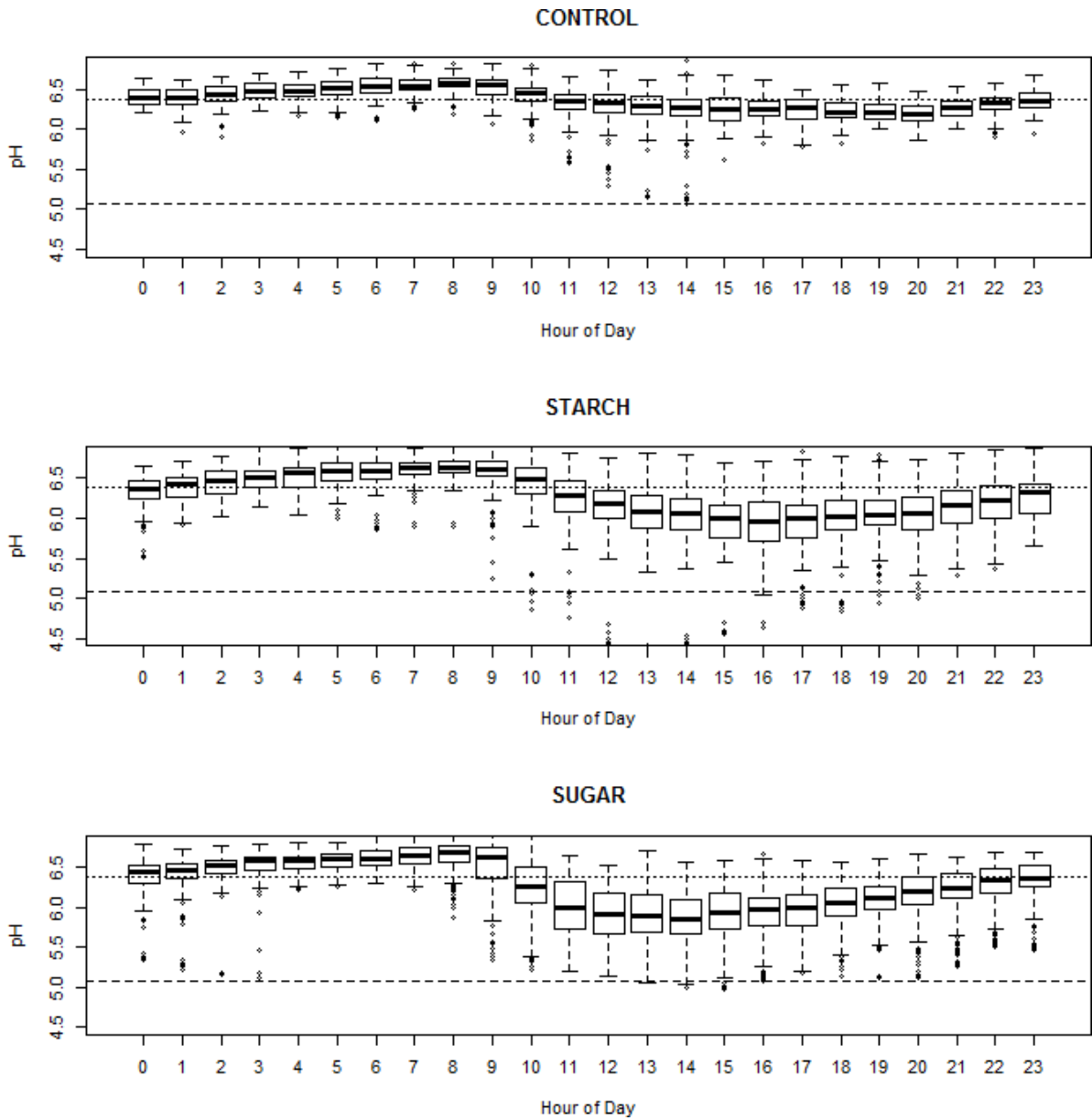


Figure 1 caption: Box and whisker plots for observed pH by hour of day, grouped according to treatment. The horizontal dotted line is the overall median value for the “CONTROL” group and the horizontal dashed line is the minimum value for the “CONTROL” group.

Figure S2 - supplementary materials.

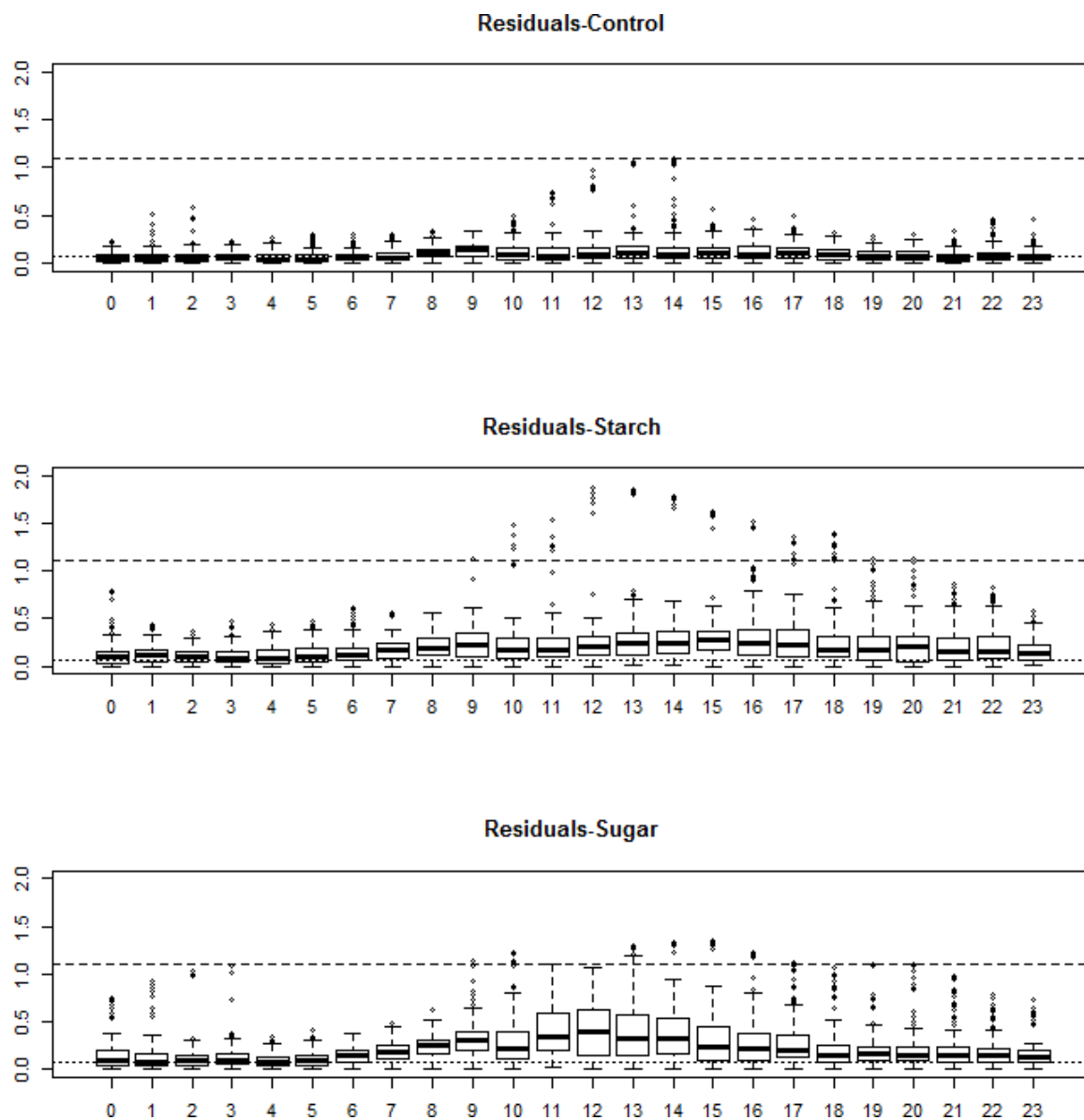


Figure 2 caption: Box and whisker shows the mean absolute residuals by hour of day and dietary treatments. The horizontal dotted line is the overall median value for the “CONTROL” group and the horizontal dashed line is the maximum value for the “CONTROL” group.