Research Institute for Farm Animal Biology

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PIGWEB

Oro-caecal transit time measured with the lactose-^{13}C-ureide breath test, in pigs fed high and low fibre diets

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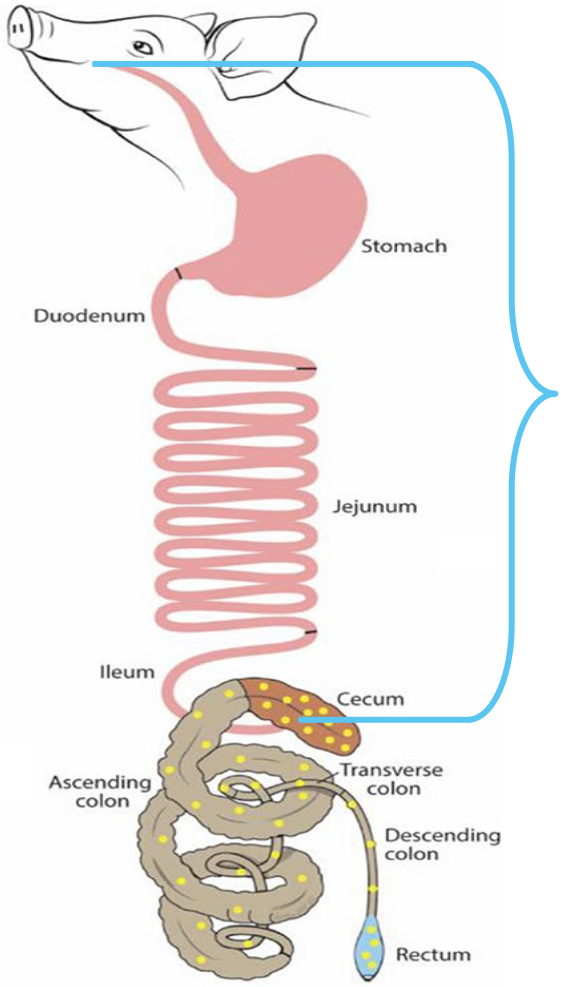
Background



Source: Schaaf CR and Gonzalez LM (2022)

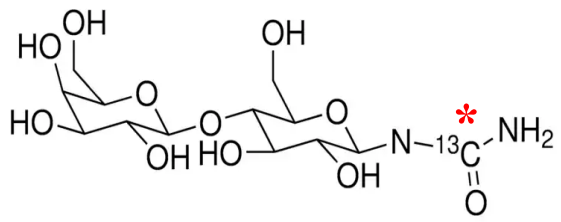
OCTT

Oro-caecal transit time:
The time elapsed for the digesta to transit from mouth to caecum

INVASIVENESS

Cannulation of different gut segments (duodenal, distal ileum or caecal)

Lactose-^{13}C-ureide



Lactose-ureide[1]

labelled with the stable isotope ^{13}C on the urea moiety (**L^{13}CU**)

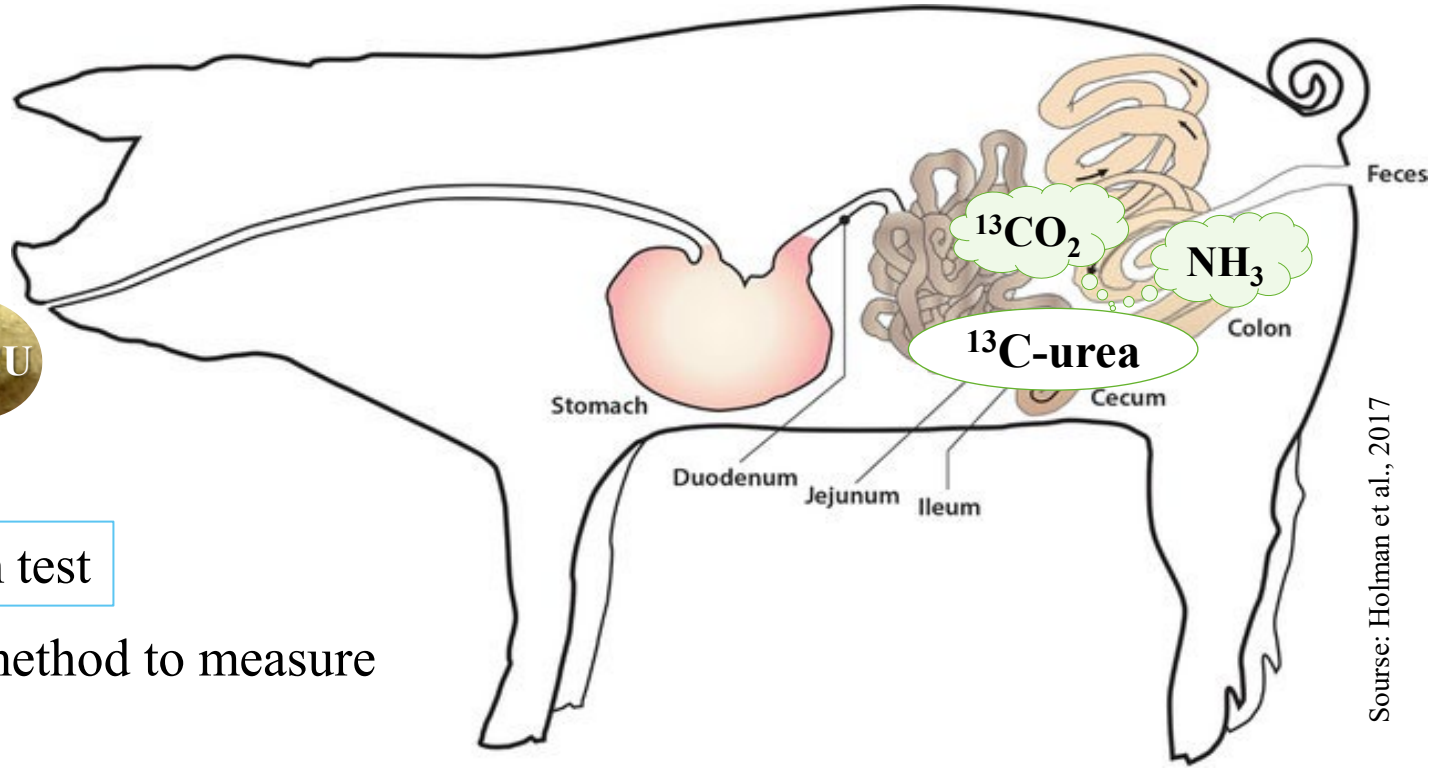
- Bacterial glucose-ureide hydrolase: Liberation of ^{13}C-urea
- Urease: NH_3 and $^{13}CO_2$
- $^{13}CO_2$ exhaled with breath

Humans[1]
Horses[2]
Rats[3]

L^{13}CU

L^{13}CU-Breath test

validated as non-invasive method to measure OCTT



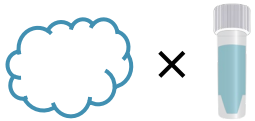
Sourse: Holman et al., 2017

[1]Geypens et al., 1999
[2]Sutton et al., 2011
[3]Uchida and Yoshida, 2009

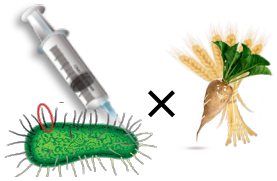
Aims

1. Measure the OCTT using L^{13}CU and $^{13}CO_2$ in breath samples

2. Measure the OCTT using L^{13}CU and $^{13}CO_2$ in saliva samples



3. Compare breath and saliva to assess the use of saliva as minimally invasive sample as a replacement for breath



4. Investigate whether measuring OCTT with L^{13}CU test reflects the effects of dietary fibre and immune stimulation (LPS) in pigs

Experimental design

NUTRITIONAL MODEL

IMMUNE CHALLENGE MODEL

Components [g/100 g fresh matter]	LF	HF
Basal diet	85.3	85.3
Soybean oil	0.2	4.0
Wheat starch	14.5	-
Lignocellulose	-	4.5
Sugar beet pulp	-	6.2

	LF	HF
LPS	LF-LPS	HF-LPS
NaCl	LF-NaCl	HF-NaCl

400 mg Lactose-ureide +

stimulate bacterial enzymes

Metabolic Crates

i.m. LPS 30 µg/kg BW NaCl control

L^{13}CU test

Dietary adaptation

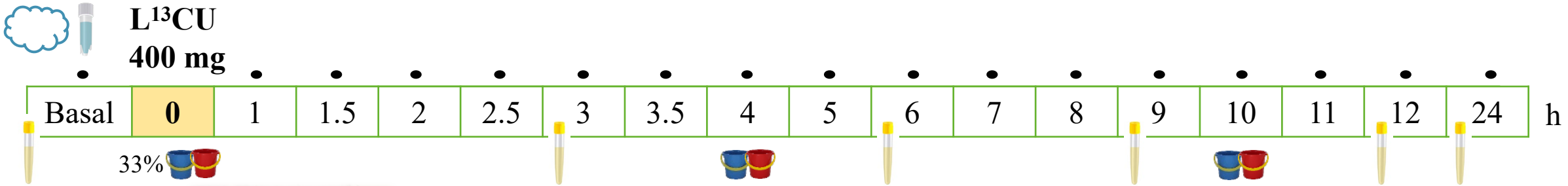
Low Fibre LF n = 22

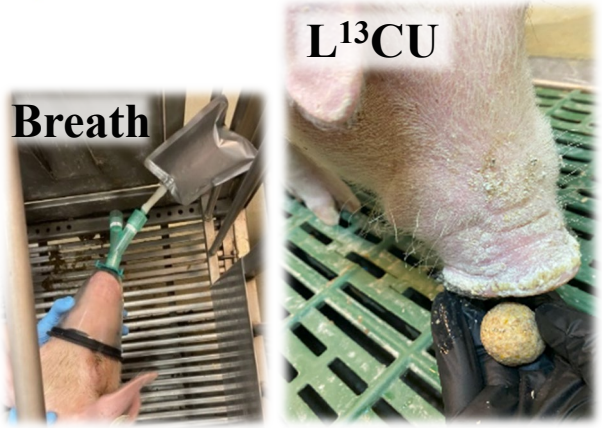
High Fibre HF n = 22

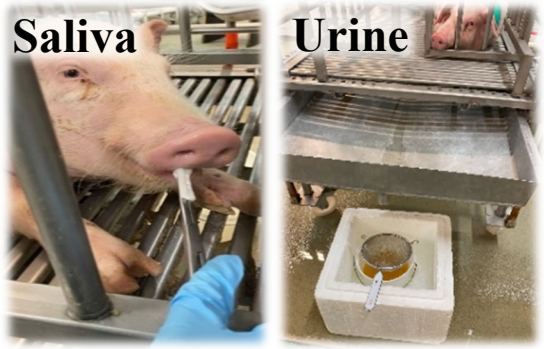
50 — 74 — 75 — **76** — Age (d)

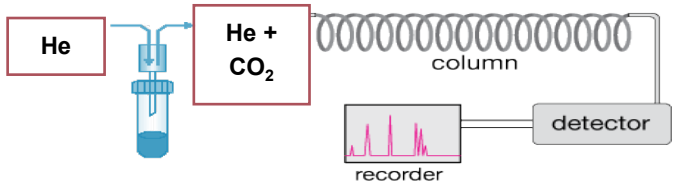
Zootechnical parameters: Body weight, Feed intake, Water intake

Lactose-^{13}C-ureide test





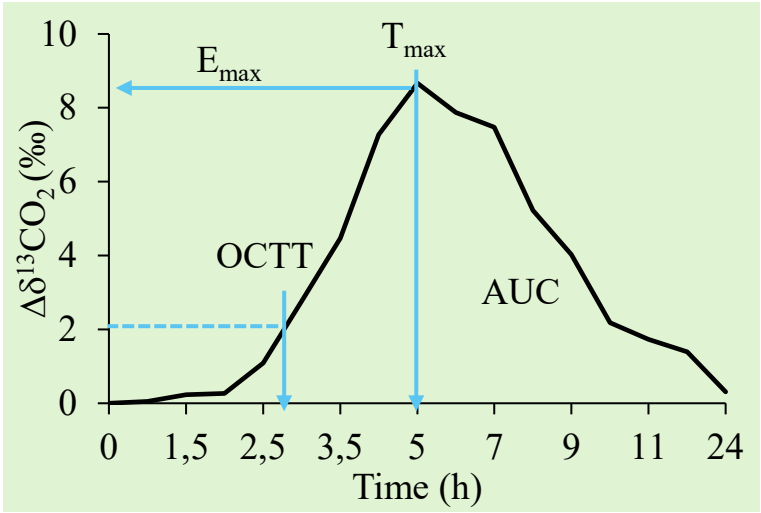




Breath/Saliva: Gas Chromatography
Urine: Elemental analyzer
+
Isotope Ratio Mass Spectrometry

→ Enrichment $\delta^{13}C$ (‰) = ^{13}C / ^{12}C against standard (PDB)

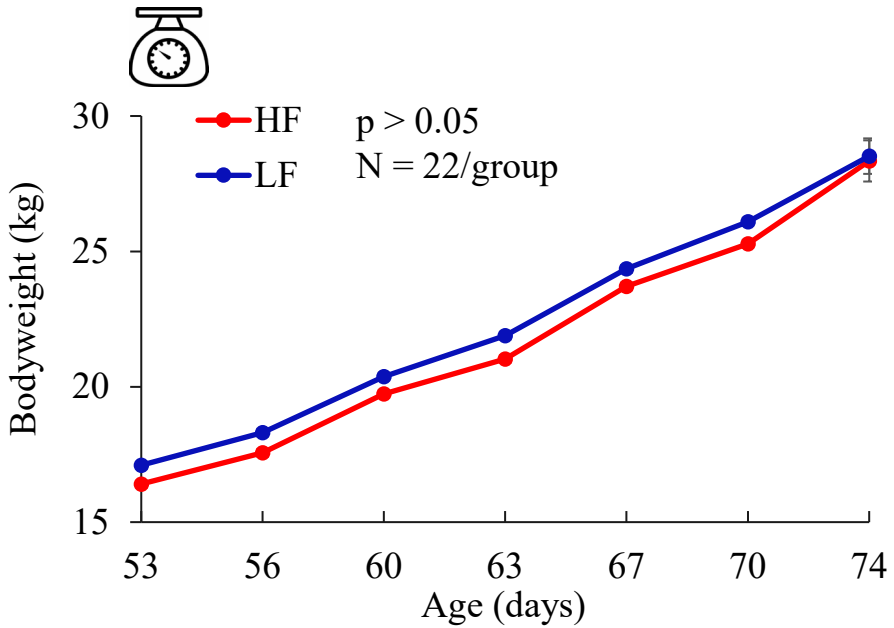
→ $\Delta\delta^{13}CO_2$ (‰) = measured enrichment – basal enrichment

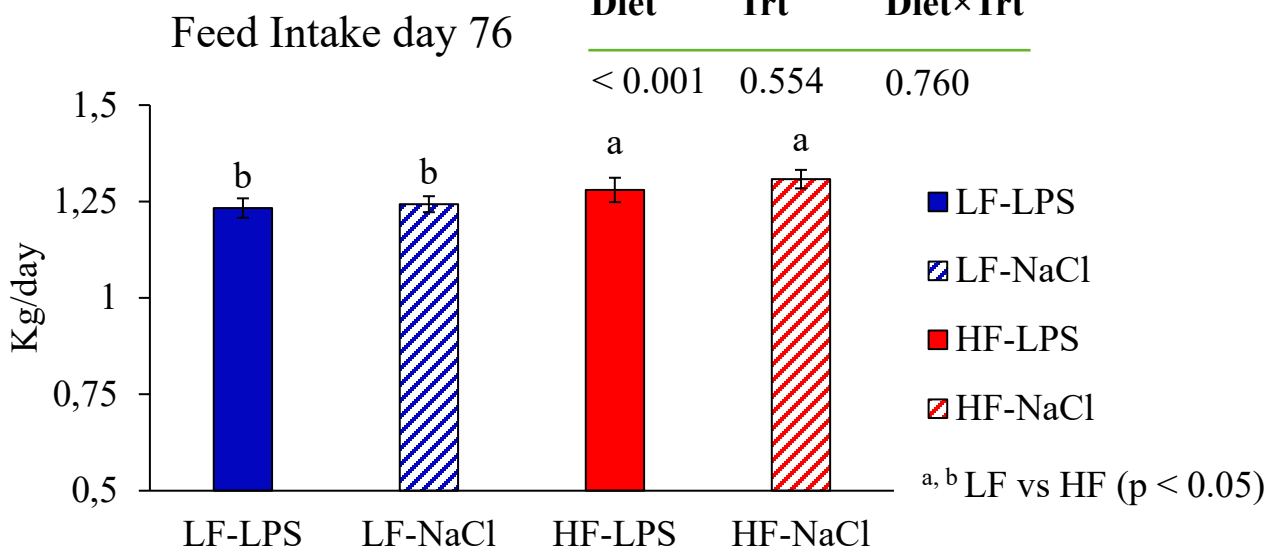


OCTT: oro-caecal transit time (time to reach 2 $\Delta\delta^{13}CO_2$)
AUC: area under the enrichment-time-curve
E_{max} : maximum enrichment
T_{max}: time to reach the maximum enrichment

Mixed model of SAS for the analysis

Zootechnical parameters



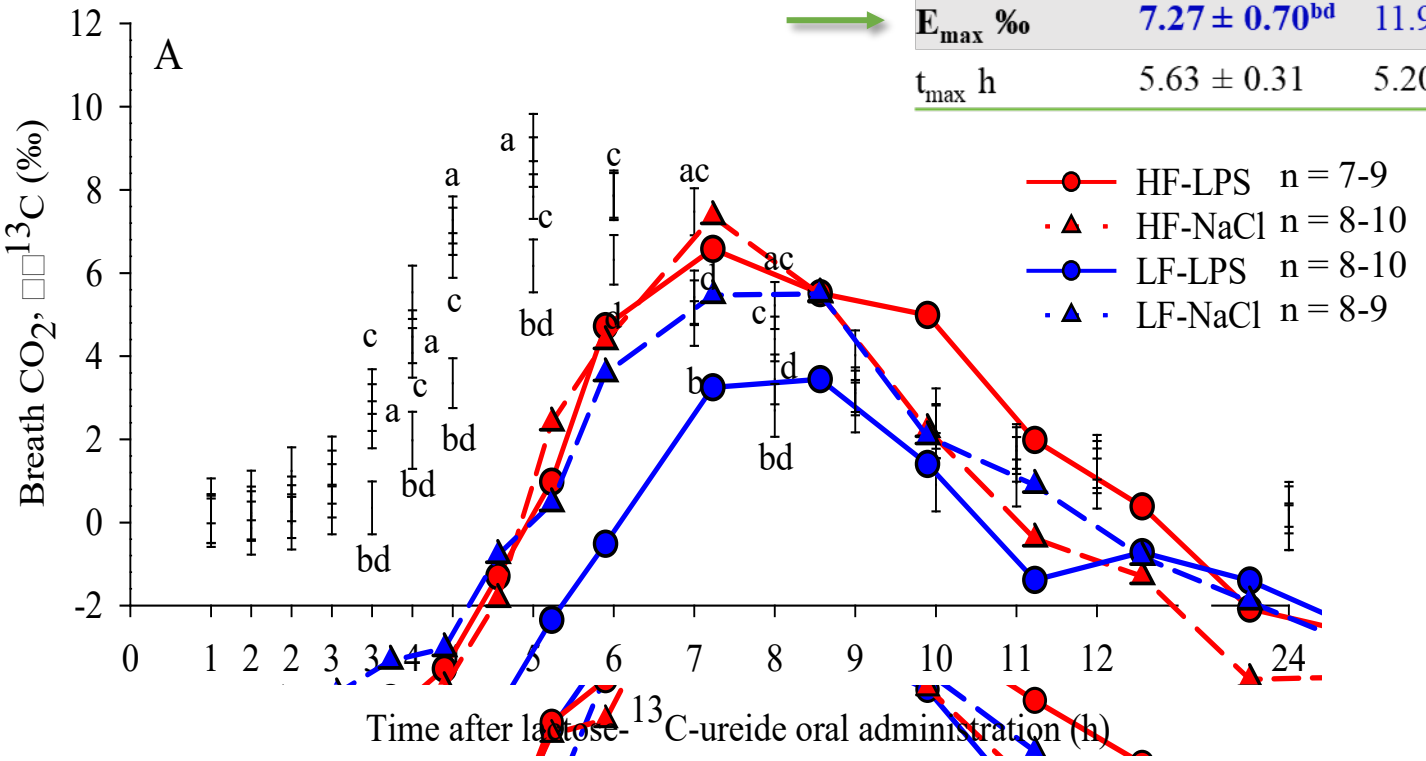


Diet	Trt	Diet×Trt
< 0.001	0.554	0.760

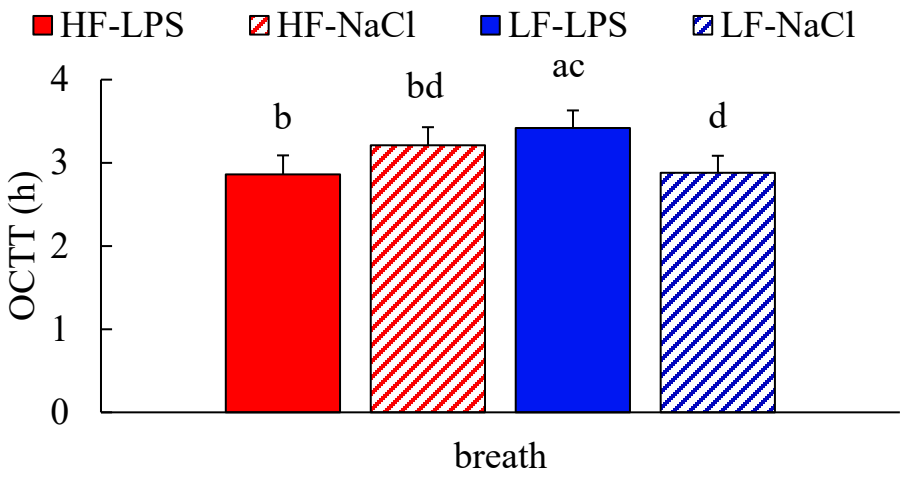
	Low Fibre		High Fibre		P-value		
	LPS	NaCl	LPS	NaCl	Diet	Trt	Diet×Trt
N	8-10	8-9	7-9	8-10			
Water intake (L/d)	3.67 ± 0.23	4.08 ± 0.52	3.56 ± 0.35	3.55 ± 0.25	0.460	0.743	0.923
Energy intake (MJ/d)	18.9 ± 0.39	19.0 ± 0.32	18.6 ± 0.46	19.0 ± 0.35	0.116	0.554	0.760

L^{13}CU test: Breath $^{13}CO_2$

	Low Fibre		High Fibre		P-value	
	LPS	NaCl	LPS	NaCl	Trt	Diet×Trt
AUC ‰·h	45.7 ± 2.9	46.7 ± 2.6	48.6 ± 2.8	46.4 ± 2.6	0.802	0.517
E_{max} ‰	**7.27 ± 0.70^{bd}**	11.9 ± 0.75^{c}	**9.68 ± 0.65^{a}**	9.86 ± 0.61^{ac}	< 0.01	< 0.01
t_{max} h	5.63 ± 0.31	5.20 ± 0.30	5.00 ± 0.33	5.16 ± 0.32	0.647	0.291



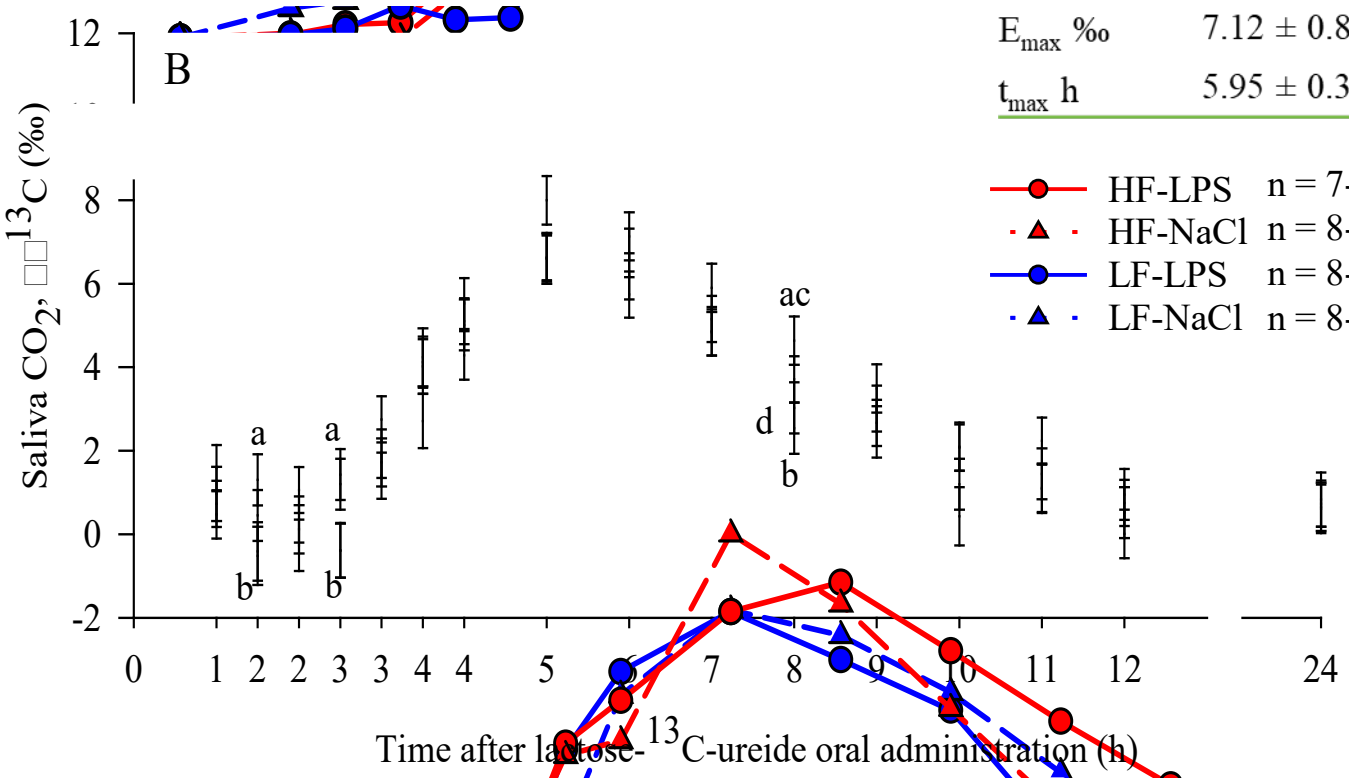
Diet	Trt	Diet×Trt
0.505	0.591	< 0.05



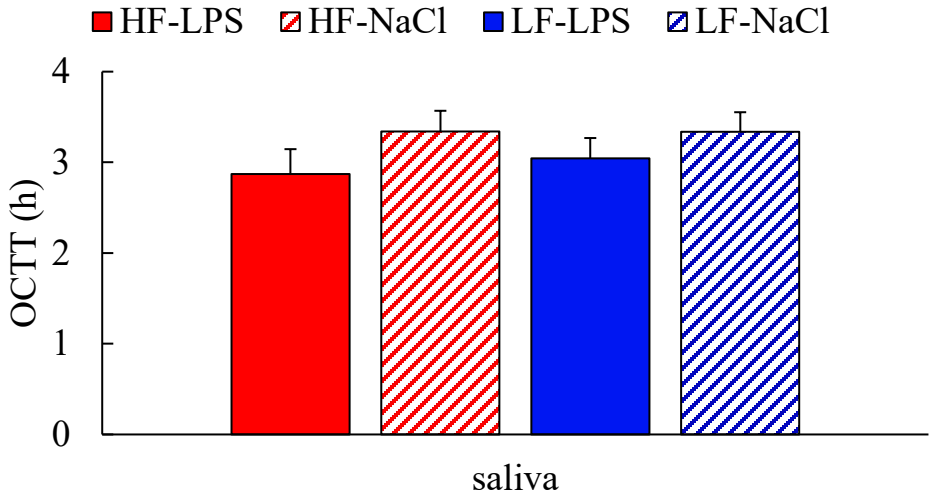
[a, b] LF vs HF, within same treatment Trt ($p < 0.05$)
[c, d] NaCl vs LPS, within same diet ($p < 0.05$)

L^{13}CU test: Saliva $^{13}CO_2$

	Low Fibre		High Fibre		P-value	
	LPS	NaCl	LPS	NaCl	Trt	Diet×Trt
AUC ‰·h	50.9 ± 4.3^{ac}	34.9 ± 4.6^{d}	40.0 ± 4.8^{ad}	40.1 ± 5.2^{ad}	0.106	0.102
E_{max} ‰	7.12 ± 0.87	7.85 ± 0.75	7.86 ± 0.80	8.69 ± 0.75	0.304	0.944
t_{max} h	5.95 ± 0.34	5.17 ± 0.41	5.19 ± 0.36	5.14 ± 0.34	0.251	0.314

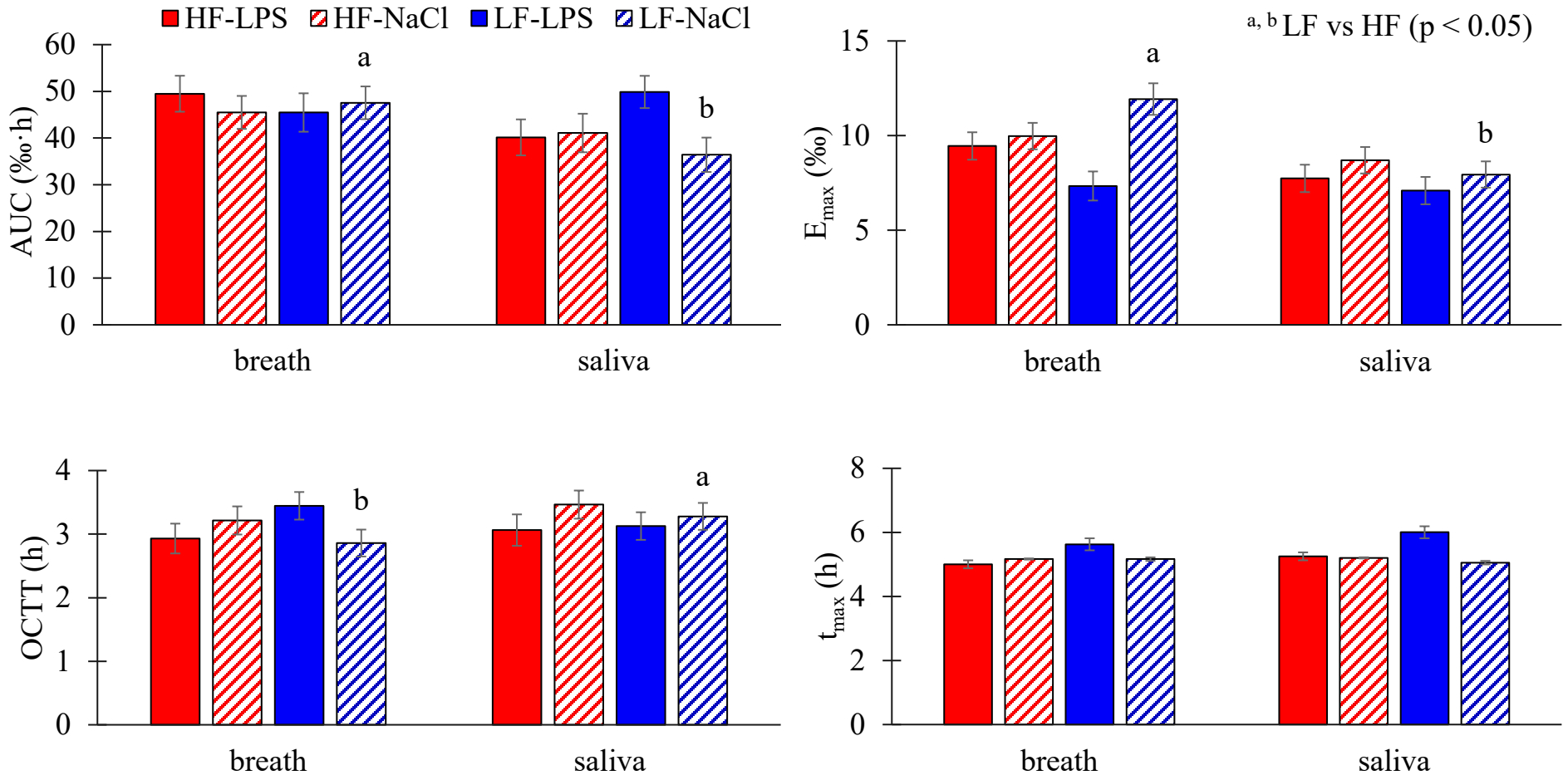


Diet	Trt	Diet×Trt
0.718	0.111	0.699

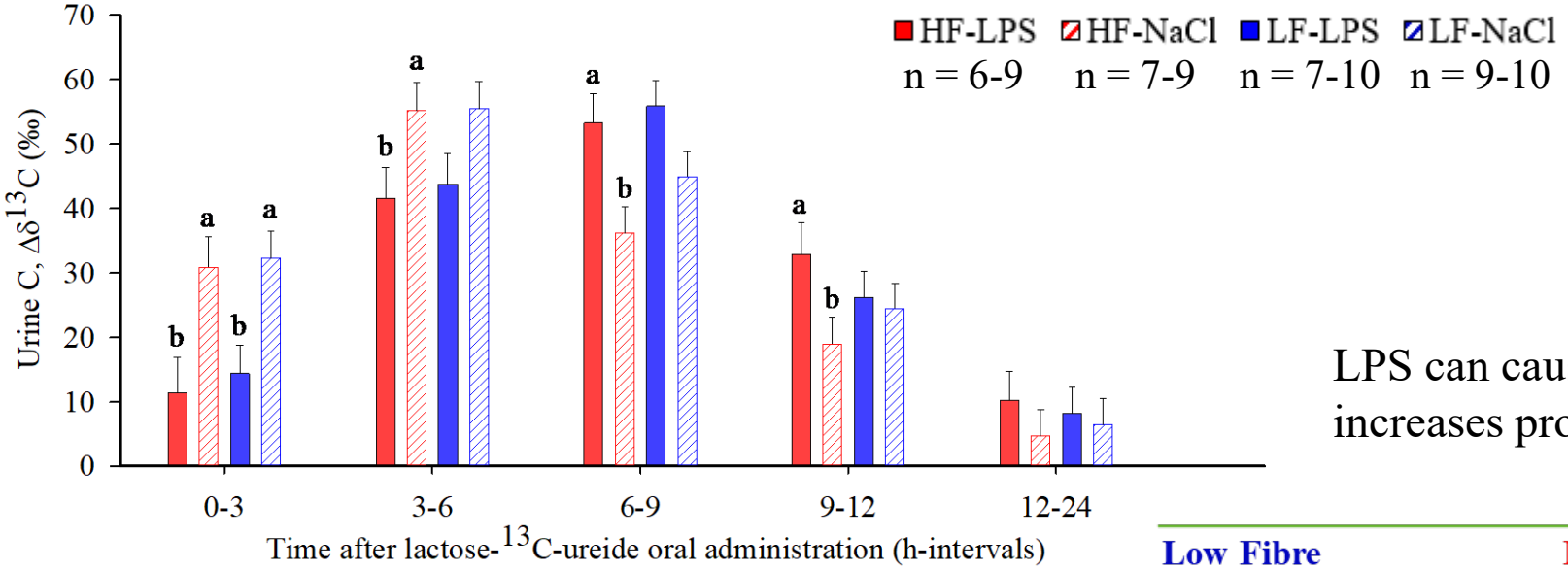


[a, b] LF vs HF, within same treatment Trt ($p < 0.05$)
[c, d] NaCl vs LPS, within same diet ($p < 0.05$)

L^{13}CU test: Breath $^{13}CO_2$ vs Saliva $^{13}CO_2$



L^{13}CU test: Urine ^{13}C



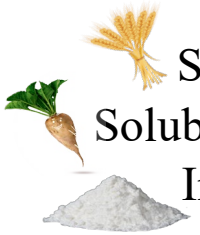
LPS can cause liver or kidney impairment and increases protein breakdown and nitrogen loss as urea[1]

	Low Fibre		High Fibre		P-value		
	LPS	NaCl	LPS	NaCl	Diet	Time	Trt
Urea mmol/L					0.635	< 0.001	< 0.001
Overnight	225 ± 24.2^{c}	106 ± 24.2^{d}	201 ± 25.5^{c}	104 ± 30.6^{d}			
0-3 h	198 ± 24.8^{c}	73.9 ± 24.7^{d}	132 ± 27.8	86.7 ± 25.5			
3-6 h	149 ± 26.2^{b}	96.0 ± 25.3	246 ± 26.1^{ac}	91.5 ± 26.8^{d}			
6-9 h	204 ± 24.2^{c}	125 ± 24.9^{d}	244 ± 25.5^{c}	134 ± 25.5^{d}			
9-12 h	133 ± 24.2	64.3 ± 26.1	149 ± 26.3	77.4 ± 26.3			

$^{a, b}$ LF vs HF, within same treatment Trt ($p < 0.05$)
$^{c, d}$ NaCl vs LPS, within same diet ($p < 0.05$)

[1]Doi et al. 2009

Discussion and conclusions



Starch (faster transit)
Soluble fibre (slower transit in the upper gut)
Insoluble fibre (faster transit in the upper gut)[1]

LPS induces delayed gastric emptying and has inhibitory effects on gastrointestinal motility (Rats/Canine/Human studies)[2]

No differences in OCTT

LF-NaCl (2.9 ± 0.2 h)

HF-NaCl (3.2 ± 0.2 h)

- Method limitation
- Fibre inclusion

Differences in OCTT

LF-LPS (3.4 ± 0.2 h)

LF-NaCl (2.9 ± 0.2 h)

HF-LPS (2.9± 0.2 h)

Fibre "protective" effect over LPS

- OCTT can be measured in pigs using L^{13}CU using breath and saliva samples
- Saliva $^{13}CO_2$ was less sensitive than breath
- The method could be a potential proxy for monitoring the gut health of pigs

[1]Ratanpaul et al., 2019
[2]De Winter and De Man, 2010

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Questions
Opinions
Comments
?

Thank you for the attention!

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Table: Urine and faeces production, ^{13}C and nitrogen balance of 76 d pigs during the L^{13}CU test[1]

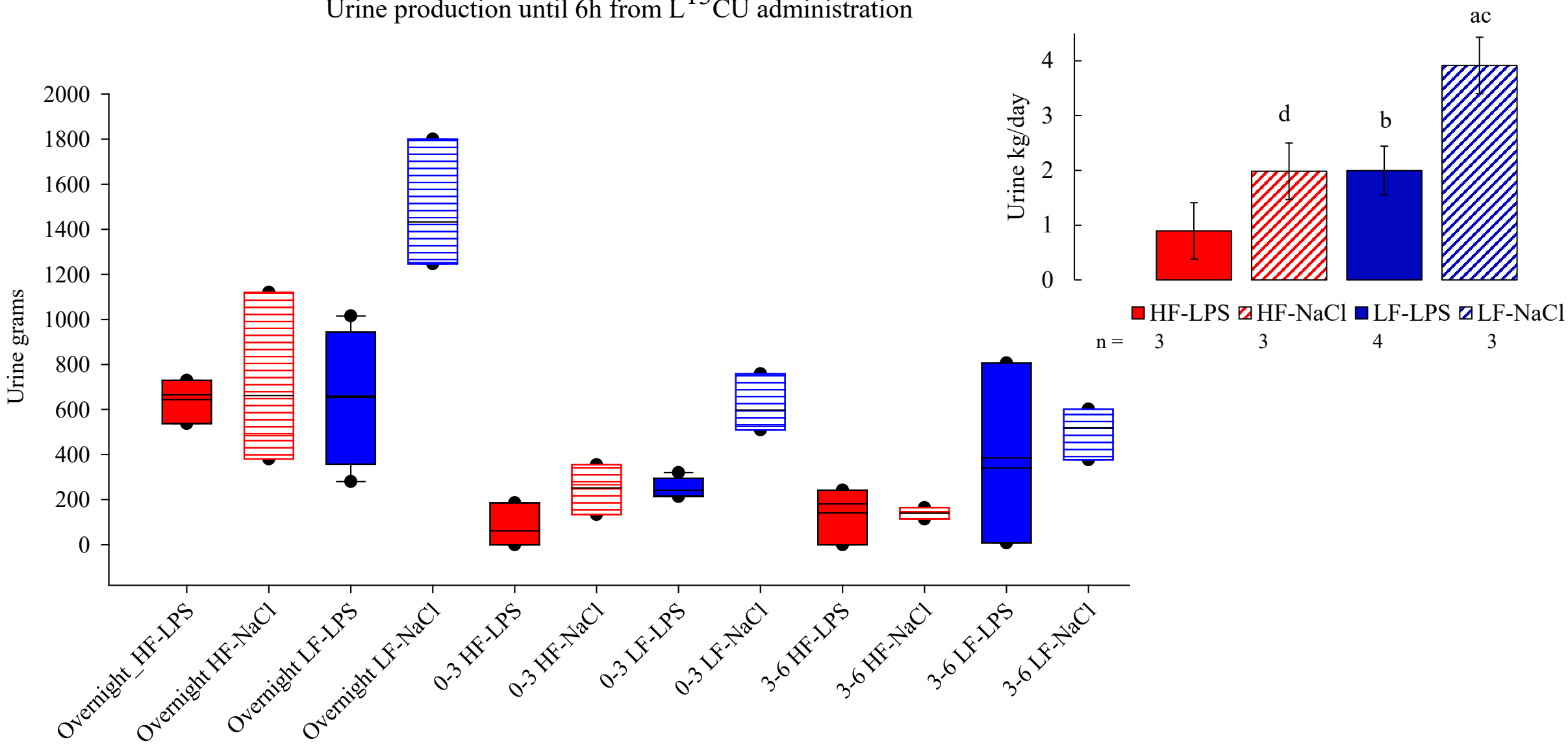
Items	Low Fibre		High Fibre		*P*-value[2]	
	LPS	NaCl	LPS	NaCl	Diet	Trt
Faeces, g/d	429 ± 61.9^{b}	577 ± 71.4^{b}	774 ± 71.4^{ad}	1089 ± 71.4^{ac}	< 0.001	< 0.01
Urine, g/d	1997 ± 446^{d}	3915 ± 515^{ac}	895 ± 515	1984 ± 515^{b}	< 0.05	< 0.05
^{13}C balance, g/d	1.68 ± 0.33	1.57 ± 0.38	1.60 ± 0.38	1.81 ± 0.38	0.829	0.889
Ingested N, g/d	31.4 ± 0.57^{b}	32.2 ± 0.66^{b}	38.6 ± 0.66^{a}	39.5 ± 0.66^{a}	< 0.001	0.196
Faecal N, g/d	5.07 ± 0.77	6.20 ± 0.89^{b}	6.97 ± 0.89	9.24 ± 0.89^{a}	< 0.05	0.081
Faecal N/Ingested N, %	16.0 ± 2.28	19.3 ± 2.63	18.1 ± 2.63	23.4 ± 2.63	0.253	0.128
Urine N, g/d	8.93 ± 0.76^{c}	6.26 ± 0.88^{d}	7.11 ± 0.88	5.91 ± 0.88	0.234	< 0.05
Urine N/Ingested N, %	28.6 ± 2.36^{ac}	19.4 ± 2.72^{d}	18.4 ± 2.72^{b}	15.0 ± 2.72	< 0.05	< 0.05
N excreted, g/d	14.0 ± 0.65	12.5 ± 0.75^{a}	14.1 ± 0.75	15.1 ± 0.75^{b}	0.087	0.744
Urine N : Faecal N	2.09 ± 0.34	1.12 ± 0.39	1.02 ± 0.39	0.65 ± 0.39	0.072	0.108

[1]Values are least-square means ± standard error of the mean: LF-LPS ***n* = 4**; LF-NaCl/HF-LPS/HF-NaCl ***n* = 3**

[2]*F*-test, Diet × Trt interaction was not significant ($P > 0.1$)

[a,b]Values with different superscripts within row differ between diets within treatment ($P < 0.05$)

[c,d]Values with different superscripts within row differ between treatments within diet ($P < 0.05$)


Urine production until 6h from L13CU administration
Urine grams
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Overnight _HF-LPS
Overnight HF-NaCl
Overnight LF-LPS
Overnight LF-NaCl
0-3 HF-LPS
0-3 HF-NaCl
0-3 LF-LPS
0-3 LF-NaCl
3-6 HF-LPS
3-6 HF-NaCl
3-6 LF-LPS
3-6 LF-NaCl
Urine kg/day
4
3
2
1
0
ac
d
b
HF-LPS
HF-NaCl
LF-LPS
LF-NaCl
n =
3
3
4
3

Faeces during L^{13}CU test