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Is gastrointestinal plasticity in king quail (Coturnix chinensis) elicited by diet-fibre or diet-energy dilution?

Sean A. Williamson
University of Wollongong, saw712@uowmail.edu.au

Stephanie Kirsten Courtney Jones
University of Wollongong, skcj542@uowmail.edu.au

Adam Munn
University of Wollongong, amunn@uow.edu.au

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Keywords

Quail, Coturnix chinensis, Phenotypic plasticity, Dietary fibre, Energy dilution, Digestive physiology, Gastrointestinal tract, Gastroliths, Gizzard

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Is gastrointestinal plasticity in king quail (*Coturnix chinensis*) elicited by diet-fibre or diet-energy dilution?

Sean A Williamson, Stephanie K Courtney Jones, Adam J Munn*

Institute of Conservation Biology and Environmental Management, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2500, Australia

Phone, +612 4221 4459; amunn@uow.edu.au

* To whom correspondence should be directed

Abstract

Phenotypic plasticity of organ size allows some animals to manage fluctuations of resource quality or availability. Here, we examined the phenotypic plasticity of the gastrointestinal tract of king quail (*Coturnix chinensis*) in a diet-fibre manipulation study. Quail were offered either a control low-fibre (high-quality) food (8.5% neutral-detergent fibre; NDF), or one of two experimental diets of higher fibre contents of 16% NDF (i.e. low quality food). To examine whether phenotypic plasticity of organ size was associated with the fibre content *per se*, or as a consequence of diluting the diet energy contents by adding fibre, one of the high-fibre feeds was 'balanced' with additional energy to match that of the low-fibre control diet. Total empty dry mass of the gastrointestinal tract was significantly heavier among birds offered the unbalanced high-fibre diet as compared with those offered the control diet, with birds offered the fibrous but energy-balanced diet having guts of intermediate size. The heavier entire-gut mass (dry) of quail offered the unbalanced high-fibre diet was associated

mainly with these birds having significantly heavier gizzards. Notably, the larger gizzard in the birds offered the unbalanced high-fibre diet was associated with marked increases their metabolisability (digestion) of diet fibre. Our findings suggest that the available energy in the diet may be more important for eliciting phenotypic changes in the gut of these herbivorous birds rather than simple physical effects of diet fibre on feed intakes or on muscular compensation to fibrous ingesta.

Key words

Quail, *Coturnix chinensis*, phenotypic plasticity, dietary fibre, energy dilution, digestive physiology, gastrointestinal tract, gastroliths, gizzard.

Introduction

Phenotypic plasticity of the avian gastrointestinal tract (gut) has been demonstrated for numerous species. For many avian herbivores the gut is especially responsive to changes in diet quality, but the physical and biochemical mechanisms that drive this plasticity are uncertain (Piersma and Lindstrom, 1997; Stark, 2005). Diet quality is important for vertebrate herbivores because they lack the ability to breakdown the hard-to-digest, fibrous components of vegetation aut-ezymatically (Barboza et al. 2009). Consequently, avian herbivores have been shown to increase the size of some intestinal organs, particularly the gizzard and paired caeca, to assist mechanical breakdown and the microbial-assisted fermentation of plant fibre that typically contain high proportions of cellulose, hemicellulose and lignin (Barboza et al. 2009). As such, a common method for investigating gut plasticity in herbivorous birds involves manipulating diet fibre levels by diluting high-quality, low-fibre feeds with increasing levels of hard-to-digest, fibrous material. In this regard, diet-dilution, and specifically diet-energy dilution, refers to the concomitant decrease in easily accessible nutrients (e.g. soluble cell contents) that accompanies any increase in the contents of hard-to-digest, fibrous material (i.e. digestible rather than gross energy contents – see Barboza et al 2009). However, to the best of our knowledge, no previous studies have been able to distinguish potential effects of diet-energy dilution from any effects associated with changes in food intake rates or as a consequence of any physical attributes that fibre might have on gut muscle. Therefore, using three novel diet formulations we isolated the effects of diet-fibre contents and energy dilution on the food intakes, metabolisability and gastrointestinal plasticity of a small herbivorous bird, the king quail (*Coturnix chinensis*).

The three diets offered to our quail (Table 1) were either a high-quality, low-fibre (LF) food containing around 8% neutral-detergent fibre (NDF; mainly cellulose, hemicellulose and

63 lignin) and around 3% acid detergent fibre (ADF; mainly cellulose and lignin), or one of two
64 high-fibre (low-quality) diets, each containing around 16% NDF and 6-7% ADF). To
65 examine whether changes in organ size were associated with the fibre content of the diets *per*
66 *se*, one of the high-fibre diets was balanced with additional energy (HFB) to match the energy
67 contents of the LF control diet, but the second high-fibre diet remained unbalanced (HFU),
68 and was therefore energy-dilute. Diets were same in all other respects (Table 1), and were
69 based on a standard poultry formulation (see methods).
70
71 **Results and discussion**
72 The first key finding of our study was that morphological adjustments of the quail gut could
73 be driven by energy-dilution effects, independent of food intakes and not solely as a
74 consequence of diet fibre. Specifically, quail offered the energy-dilute HFU diet had heavier
75 guts (entire dry mass) than those offered higher quality LF, but not the high fibre but nutrient
76 balanced (HFB) diets (Table 2). These differences were driven mainly by the significantly
77 heavier gizzards of the HFU-fed birds (wet and dry masses), being 1.4 times those of the LF-
78 fed quail, and 1.2 times heavier than the HFB-fed birds, though the latter group's gizzards
79 were not significantly different from either the LF- or HFU-fed birds. These results are
80 suggestive of a graded response in organ plasticity, whereby the need for a larger gizzard by
81 the HFB-fed birds was apparently tempered by access to more easily accessible energy
82 content of their diet.
83
84 Importantly, the larger gizzards of the HFU-fed birds apparently allowed them to maintain
85 body mass and body condition (fat mass) throughout the entire experiment (Table 3). By the
86 end of the experiment there were no significant differences in the abdominal fat masses
87 between the LF- or HF-fed quails (i.e. HFB or HFU; Table 2). Likewise, the HFU-fed quail

88 maintained feed intakes (dry and organic matter) comparable to those of LF-fed and HFB-fed
89 birds (Table 1), in support of Starck's (1999) suggestions that vertebrate gut-plasticity may be
90 largely independent of food intake rates. Additionally, we provide the first experimental
91 evidence that diet energy composition (or energy dilution) may be critically important for
92 eliciting phenotypic plasticity of the vertebrate gut rather than the fibre content *per se*.
93
94 The second key finding of our study was that the HFU-fed quail had markedly higher
95 metabolisability of plant fibres (NDF and ADF) compared with those offered the LF or
96 nutrient-balanced HF diets (Table 3). Although the apparent metabolisability of organic
97 matter by the LF-fed quail were on average higher than those by the HFB and HFU-fed birds,
98 , these differences were relatively minor compared with strikingly high levels of fibre
99 digestion by the HFU-fed birds. Overall, the HFU-fed birds apparently metabolised 42% of
100 ingested NDF, and 21% of ingested ADF, levels that were around twice those for the LF- and
101 HFB-fed birds (Table 3).
102
103 The main sites for microbial-assisted fermentation in herbivorous birds are the paired caeca,
104 and marked increases in caecal-mass have been observed in numerous bird species when
105 feeding on high-fibre diets (e.g. Moss, 1974). However, the generally heavier paired-caeca of
106 our HFU-fed quail was not statistically significantly different from that of the LF- or HFB-
107 fed birds, although these data were quite variable (Table 2). Moreover, it is entirely possible
108 that the differences in caecal masses for the HFU-fed birds were biologically relevant,
109 particularly when other intestinal features are considered. For example, the HFU-fed birds
110 tended also to have heavier proventriculus tissue (wet and dry masses; Table 2). The avian
111 proventriculus is proximal to the gizzard (or ventriculus), and is the main acid-secreting
112 organ, but there is evidence that increased acid digestion, along with greater mechanical

113 action in the gizzard, improves fibre degradation (Svihus, 2011). Moreover, mechanical
114 action of the avian gizzard is boosted by gastroliths (or gizzard rocks/stones), and these
115 tended to be more numerous ($P = 0.06$) and have a heavier overall mass (0.14) in the HFU-
116 fed birds (Additional Item Figure A1). It is also possible that changes to the caecal microbial
117 community-composition or population sizes could have affected higher fibre metabolisability
118 by the HFU-fed birds. Nonetheless, it is apparent that the high metabolisability of fibre by the
119 HFU-fed birds aided their maintenance of body-condition despite the challenging diet. As
120 such, we present tangible evidence for improved fibre digestion in an avian herbivore
121 associated with morphological plasticity of the gut.
122
123 Presumably, the larger gizzard of the HFU-fed quails facilitated mechanical and fermentative
124 digestion in our quail by improving fibre particle-size reduction, with the aid of gastroliths. In
125 this regard, food bulkiness may present an important mechanism activating phenotypic
126 changes of the vertebrate gut. Other studies have demonstrated that increases of structural
127 complexity of diets (i.e. increases in hard-to-digest fibre) increase the volume of gizzard
128 digesta, in addition to increases of gizzard tissue mass (Svihus, 2011). Larger particles are
129 generally retained in the avian gizzard until they are reduced below a threshold particle-size.
130 For example, in domestic chickens, particles typically pass from the gizzard only once they
131 are reduced to 0.5 -1.5mm (Moore, 1999). Such a threshold particle-size for passage from the
132 king quail gizzard is uncertain, but it is worth noting that our HFU-fed birds' gizzards
133 contained 1.3 times the wet-contents of the LF-fed birds; the values being 5.7 $\pm$ 0.1 g and 4.3
134 $\pm$ 0.1 g for HFU- and LF-fed birds respectively (Tukey's HSD, $P < 0.05$). Furthermore, the
135 HFB-fed birds' gizzard masses (wet and dry; Table 2) and wet contents (4.6 $\pm$ 0.2 g) were
136 intermediate between the LF- and HFU-fed birds, suggesting that food bulk or particle size
137 had some effect on gizzard plasticity. Nonetheless, our central conclusion is that, in addition

to any textural, particle-size or fibre-bulk associated effects, phenotypic plasticity of the avian gut can be elicited by the energy composition of the diet offered, or that of the subsequent digesta and absorbta.

Methods and materials

Ethics

All experimental procedures were approved by the University of Wollongong's Animal Ethics Committee (Protocol No: AE11/15), in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Housing and animal management

Female king quail ($N = 18$ sexually mature, 2-3 year olds; *Coturnix chinensis*) were obtained from a commercial supplier (Andrew's Quail and Pet Palace, Smithfield, New South Wales, Australia). All quail were held at the Ecological Research Centre (ERC) at the University of Wollongong. Quails were housed individually in mesh-floored plastic cages (30 x 30 x 30 cm) and excreta were collected under each cage using a tray lined with non-stick baking paper. Animal were housed in a temperature controlled facility (22°-24°C) at 50-60% relative humidity and 14:10 h light:dark photoperiod (lights on at 0600h; full-spectrum UV fluorescent bulbs). All quail were acclimated to housing and regular husbandry procedures (e.g. handling and weighing, daily feed checks and changes, excreta collection) for three weeks prior to experimentation. Quail were weighed (±0.1g) every three days throughout acclimation and experimental periods.

160

161 **Feeding trials**

162 All diets were prepared by The Poultry Research Foundation, The University of Sydney,
163 Australia (Table 1). A standard low-fibre (LF) poultry feed contained 8.5% NDF (mainly
164 hemicelluloses, cellulose and lignin) and 3% ADF (mainly cellulose and lignin) provided all
165 animals with a consistent acclimation diet, and presented a control diet through the
166 experimental period. Two additional diets were used during the experimental period, each
167 containing higher fibre contents of 16-17% NDF and 6-7% ADF (Table 1). One of the high
168 fibre diets was 'balanced' (i.e. high-fibre balanced; HFB) with corn oil to match the
169 metabolisable energy contents of the LF diet (Table 1). The second high fibre diet was not
170 energy-balanced and was therefore energy diluted, or 'unbalanced' (i.e. high-fibre
171 unbalanced; HFU). Aside from differences in total fibre (NDF and acid-detergent fibre;
172 ADF), diets were comparable in all other respects, particularly dry matter, organic matter and
173 nitrogen contents (Table 1).

174

175 Following acclimation animals were randomly assigned to one of the three diets; LF
176 (control), HFB or HFU. For those offered HFB or HFU, transition to the treatment diet
177 occurred incrementally by diluting the LF diet with 50%, 70% and 100% of the treatment diet
178 over three days, respectively. Once fully transitioned, quail remained on their respective diets
179 for 14 days (N= 18 quail; n= 6 per treatment), during which daily feed intake (to ± 0.01 g).
180 Excreta were collected every three days on pre-weighed sections of non-stick baking paper
181 (Castaway easy-bake; Packaging Direct, Wollongong). Samples of feed offered and complete
182 excreta were frozen stored at -20°C.

183

184 **Sample analyses**

185 Samples of feed offered and excreta were thawed, thoroughly mixed and subsamples (ca. 1-2
186 g) from each quail bulked individually for the last nine days of the feeding trial. Bulked
187 excreta and feed subsamples (ca. 1-2 g) were then oven-drying (forced convection) at 55°C
188 until constant mass. Further subsamples (approximately 25% by weight) were dried at 103°C
189 until constant mass to determine complete dry matter (DM). Dry feed and excreta were
190 ground using a Wiley Mill (0.5 mm screen; Thomas Scientific, Wiley Mini Mill 3383-L40,
191 Swedesboro, NJ, USA). Subsamples (ca. 0.5 g) of ground DM were ashed at 600°C for five
192 hours in a muffle furnace (Model LCF15-12, LABEC Laboratory Equipment Pty Ltd,
193 Marrickville, NSW) to determine organic matter (OM; i.e. DM-ash).
194
195 Fibre contents of feed and excreta were determined using an ANKOM Fibre Analyser (Model
196 A220, ANKOM Technology Corp., Macedon, NY, USA). Subsamples (ca. 0.5 g) of feed and
197 excreta dried at 55°C were analysed in duplicate for NDF and ADF content the sequential
198 filter-bag technique. Prior to neutral-detergent digestion, samples were treated with 1ml of
199 heat-stable amylase (Sigma A – 3306; Sigma Aldrich, Sydney) for 80 min to remove starch,
200 and sodium sulphite and decalin were omitted from the neutral-detergent procedure (Van
201 Soest et al., 1991).
202
203 Subsamples of ground, dried (at 103°C) feed and excreta were analysed for gross energy
204 content by combusting duplicate subsamples (0.5 g) in an automatic adiabatic bomb
205 calorimeter (Gallenkamp, CBA-305, Gallenkamp and Co. Ltd, UK; calibrated every 15
206 samples using a benzoic acid standard), and total nitrogen content by combusting duplicate
207 subsamples (200 ± 10 mg) using a Leco CNS-2000 combustion analyser (Leco Inc. St
208 Joseph, Michigan, USA).

209

210 **Food intake and apparent metabolisability**

211 Apparent metabolisability (%) of diet components (e.g. dry matter, energy) was calculated as:

212 $$[(\text{Intake} - \text{Excreta}) / \text{Intake}] \times 100, \quad (1);$$

213 where intake and excreta are in g day^{-1} and contents are per unit of DM or OM (Barboza *et al.*

214 2009).

215

216 **Organ morphology**

217 At the end of each feed trial period quail were euthanized by CO_2-asphyxiation followed by

218 cervical dislocation and macroscopic dissections performed immediately. The gastrointestinal

219 tract was removed and cleared of mesentery and fat. Organs (liver, crop, proventriculus,

220 gizzard, small intestine, right and left caeca, and rectum-cloaca) were separated from the

221 entire gut and weighed (±0.001g) prior to being emptied of contents, rinsed with

222 physiological (0.9%) saline, and re-weighed to determine empty wet-mass. Organ lengths

223 were measured using electronic calipers (precision 0.01mm). Gizzard contents were collected

224 and stored frozen at -20°C for later analysis (contents of the gizzard for one animal from the

225 HFU group was inadvertently discarded). Organs (excluding liver) liver dried (forced

226 convection) to constant mass at 95°C.

227

228 **Statistics**

229 Values presented are means ± standard deviation (SD). We used an analysis of variance

230 (ANOVA) to compare across diets. Assumptions for ANOVA were tested using the Ryan-

231 Joiner test for normality and Bartlett's test for homogeneity of variances. To meet the

232 assumptions for ANOVA some data were log-transformed (ADF intake, gizzard dry mass),

233 and all proportional data were arcsine transformed. Some data sets could not be transformed

to meet ANOVA assumptions (caecal wet mass, and entire-gut dry mass) and non-parametric Kruskal-Wallace tests were in these cases. Significant differences detected by ANOVA or Kruskal-Wallace ($P \leq 0.05$) were further explored using a Tukey's Honest Significant Difference (HSD) *post hoc* tests. We used z-tests to determine whether there were significant changes in quail body mass (as a proportion of initial mass compared with a hypothetical change of zero). All analyses were performed using Minitab for Windows (version 15.1.30.0; Minitab Australia).

Acknowledgements

All experimental procedures were carried out under approval from the University of Wollongong Animal Ethics Committee (AE11/15), in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Thanks to Aaron Cowieson and Joy Gill (Poultry Research Foundation, University of Sydney) for diet formulations, Professor Mike Thompson (School of Biological Sciences, University of Sydney) and William Foley (Research School of Biology, Australian National University) for access to facilities and assistance with analysis of samples. Sincere thanks to Tobias Wang and an anonymous reviewer for their insightful and helpful comments and reviews, it is much appreciated.

Competing interests

The authors declare no competing interests.

Contributions

AM, SW and SKCJ devised the experiment, SW and SKCJ performed the experiment, SW performed sample preparation and analysis, SW and AM analysed the data, and AM and SW wrote the manuscript.

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286 **Tables**

287 Table 1: Formulations and contents for the low fibre (LF), high fibre balanced (HFB) and the

288 high fibre unbalanced (nutrient diluted; HFU) offered to king quail.

	LF	HFB	HFU
Contents as fed (%)			
Wheat – feed	72.5	44.6	54.4
Soybean meal	15.2	13.2	11.4
Wheat – bran	-	25	25
Corn oil	2.1	7.1	1.6
Salt	0.14	0.17	0.11
Sodium bicarbonate	0.21	0.17	0.16
DL Methionine	0.08	0.08	0.06
Lysine HCl	0.02	-	0.02
Limestone	8.8	8.8	6.6
Dicalcium phosphate	0.77	0.77	0.58
Vitamin premix	0.2	0.2	0.2
Composition (mean ± SD)			
Dry matter (DM; %)	92.1 ± 0.2	92.0 ± 0.3	92.3 ± 0.3
Organic matter (OM; %)	85.5 ± 0.2	81.7 ± 0.3	85.2 ± 0.2
Gross energy (kJ g^{-1} OM)	16.4 ± 0.2	18.5 ± 0.8	17.2 ± 0.4
#Metabolisable energy (kJ g^{-1} OM)	11.5 ± 0.6	10.8 ± 1.0	11.5 ± 0.7
Nitrogen (% OM)	3.07 ± 0.10	2.96 ± 0.09	3.01 ± 0.10
Neutral detergent fibre (% OM)	8.5 ± 0.5	15.6 ± 0.5	16.7 ± 2.5
Acid detergent fibre (% OM)	3.0 ± 0.0	5.9 ± 0.2	6.9 ± 0.1

289 Note: #Estimated post-hoc based on data presented in Table 1.

290 Table 2: Mean (± SD) organ, abdominal fat and liver masses from king quail offered low
291 fibre (LF; n = 6), high fibre balanced (HFB; n = 6), and high fibre unbalanced (HFU; n = 6)
292 diets.
293

	LF	HFB	HFU	Diet F or H[#]	Diet *P*
Entire gut					
Wet (g)	2.54 ± 0.61	2.86 ± 0.41	3.16 ± 0.43	2.36	0.13
[#]Dry (mg)	691.5 ± 59.0^{A}	781.5 ± 17.1^{A,B}	912.8 ± 43.4^{B}	9.06	0.01
Crop					
Wet (mg)	68.7 ± 15.4	85.5 ± 28.4	103.3 ± 22.1	3.52	0.06
Dry (mg)	16.7 ± 5.5	20.3 ± 7.5	23.8 ± 7.7	1.60	0.24
Proventriculus					
Wet (mg)	179.0 ± 23.9	202.1 ± 32.0	228.3 ± 50.1	2.66	0.10
Dry (mg)	43.7 ± 6.6	49.3 ± 7.8	56.8 ± 12.2	3.10	0.08
Gizzard					
Wet (g)	1.16 ± 0.11^{X}	1.39 ± 0.28^{X,Y}	1.64 ± 0.21^{Y}	7.79	0.005
Dry (mg)	340.2 ± 27.0^{A}	404.2 ± 93.5^{A,B}	473.3 ± 48.2^{B}	6.76	0.008
Small Intestine					
Wet (mg)	808.0 ± 201.9	896.9 ± 231.8	1113.3 ± 244.1	2.88	0.09
Dry (mg)	235.0 ± 34.2	247.0 ± 67.0	289.8 ± 62.0	1.57	0.24
Caeca					
[#]Wet (mg)	137.8 ± 9.9	143.6 ± 51.3	171.3 ± 36.5	4.99	0.08
Dry (mg)	39.0 ± 5.5	41.5 ± 17.0	48.3 ± 13.4	0.84	0.45
Rectum-Cloaca					
Wet (mg)	77.7 ± 14.3	78.8 ± 23.0	88.4 ± 14.7	0.65	0.54
Dry (mg)	17.0 ± 4.9	19.2 ± 6.1	20.7 ± 4.2	0.77	0.48
Liver (wet; g)	1.19 ± 0.16^{A,B}	1.30 ± 0.15^{B}	1.50 ± 0.20^{A}	4.8	0.02
Abdominal Fat (wet; g)	1.07 ± 0.68	1.04 ± 0.23	0.73 ± 0.23	1.0	0.39

294 Note: Within a row, means (± SD) bearing different superscripts are significantly different (^{A,}
295 ^{B} $P < 0.05$; $^{X, Y}$ $P < 0.001$). [#]Kruskal-Wallace H-statistic (see Methods).
296

297 Table 3: Mean (± SD) intakes and metabolisability by king quail offered low fibre (LF; n =
298 6), high fibre balanced (HFB; n = 6), and high fibre unbalanced (HFU; n = 6) diets.
299

	LF	HFB	HFU	Diet F	Diet *P*
Body mass					
Initial (g)	52.8 ± 4.8	50.9 ± 2.7	50.8 ± 3.8	0.5	0.62
Change (% initial)	- 0.9 ± 5.2	5.4 ± 6.8	2.8 ± 5.0	1.8	0.19
Dry matter					
Gross intake (g d^{-1})	6.56 ± 0.38	6.55 ± 0.61	6.82 ± 1.03	0.27	0.76
Metabolisability (%)	72.9 ± 4.8^{A}	61.4 ± 4.7^{B}	68.7 ± 2.9^{A}	11.2	0.001
Organic matter$^{\#}$					
Gross intake (g d^{-1})	5.91 ± 0.35	5.75 ± 0.54	6.14 ± 0.92	0.55	0.59
Metabolisability (%)	76.6 ± 3.9^{A}	66.9 ± 4.7^{B}	70.68 ± 2.2^{B}	10.1	0.002
Energy					
Gross intake (kJ d^{-1})	107.3 ± 6.3	121.2 ± 11.3	117.2 ± 17.6	1.95	0.18
Metabolisability (%)	74.7 ± 4.3^{A}	68.4 ± 4.0^{B}	71.8 ± 2.5^{B}	4.28	0.034
Nitrogen					
Gross intake (mg d^{-1})	201.3 ± 11.8	194.2 ± 18.1	205.4 ± 30.9	0.41	0.67
Metabolisability (%)	37.3 ± 10.1	28.0 ± 12.0	29.0 ± 5.3	1.71	0.21
Neutral detergent fibre					
Gross intake (mg d^{-1})	557 ± 32^{A}	902 ± 84^{B}	1088 ± 164^{C}	59.9	$<1x10^{-4}$
Metabolisability (%)	24.4 ± 17.4^{A}	19.0 ± 11.0^{A}	48.4 ± 11.4^{B}	7.99	0.004
Acid detergent fibre					
Gross intake (mg d^{-1})	174 ± 10^{X}	304 ± 14^{Y}	345 ± 52^{Y}	66.8	$<1x10^{-4}$
Metabolisability (%)	14.9 ± 21.7^{A}	9.8 ± 12.7^{A}	41.8 ± 13.0^{B}	6.65	0.008

300 Note: Within a row, means bearing different superscripts are significantly different ($^{A, B, C}P \leq$
301 0.05, $^{X, Y}P < 1x10^{-4}$). $^{\#}$Organic matter = dry mass – ash (see methods).
302