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Caecal abnormality in a layer hen (*Gallus gallus forma domestica*) not accompanied by deficits in digestive performance or egg productivity

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Caecal abnormality in a layer hen (*Gallus gallus forma domestica*) not accompanied by deficits in digestive performance or egg productivity

Abstract

We report a case of a layer hen (*Gallus gallus forma domestica*) with deviation in the morphology of the caecum, and unique opportunity to investigate the digestive performance of the animal compared with normal hens. In a study investigating digestive and reproductive performance, an atypical caecal arrangement was found in a hen that was unremarkable in regards to body mass, digestive performance and egg productivity in comparison to other hens fed a similar diet. Examination of the gastrointestinal tract revealed a singular tubular outgrowth from the ileo-caecal junction, rather than the typical paired outgrowths. The single caecal duct bifurcated into two separate blind-ended sacs. Similar caecal deviations have been described in adult and juveniles, but no indications of animal performance were reported in these cases. We conclude that if the presence of an abnormal caecal arrangement reduces digestive abilities they were not obvious, and some compensatory mechanism/s may exist. Alternatively, the abnormal caecal arrangement of our hen might function adequately, such that no compensation in feed intakes or reduced egg productivity was required or observed.

Keywords

Caecal malformation, nutrition, organogenesis, poultry, physiology

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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1 Caecal abnormality in a layer hen (*Gallus gallus* forma domestica) not accompanied by
2 deficits in digestive performance or egg productivity.

3

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13 Key words: Poultry, Physiology, Organogenesis, Nutrition, Caecal malformation

14 **Abstract**

15 We report a case of a layer hen (*Gallus gallus* forma domestica) with deviation in the
16 morphology of the caecum, and unique opportunity to investigate the digestive
17 performance of the animal compared with normal hens. In a study investigating digestive
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26 compensatory mechanism/s may exist. Alternatively, the abnormal caecal arrangement of
27 our hen might function adequately, such that no compensation in feed intakes or reduced
28 egg productivity was required or observed.

29

30 **Introduction**

31 Caecal abnormalities in poultry are not common, with the reported incidences being as low
32 as n= 6 in 13,483 birds and n = 3 in 35,000 birds (see Grewal et al., 1976; Grewal and Brar,
33 1989). Documented cases have typically been seen in birds associated with other maladies
34 (Grewal et al. 1976). However, in cases where no other malady is obvious, the consequences
35 to animal nutrition of these caecal malformations are uncertain. We report a unique
36 opportunity to investigate a case of deviation in morphology of the caecum in a layer hen
37 (*Gallus gallus* forma domestica) that was discovered during an investigation into the

38 digestive and production performance of layer hens in response to different diets (Courtney
39 Jones et al. *In press*). As such, we were able to compare post-hoc the nutritional and
40 production performance of the single layer hen displaying caecal abnormality with that of
41 normal hens.

42

43 **Materials and methods**

44 *Animals*

45 All hens used in this project were aged > 18 months in production (estimated age of two
46 years), and we followed their performance in response to increased dietary fibre content in
47 a related study (Courtney Jones et al. *In press*). For the purposes of the comparison
48 described here, our single abnormal hen (discovered during post-mortem evisceration) was
49 part of a larger treatment group offered a high-fibre diet balanced with corn oil to match
50 energy contents of a standard low-fibre layer diet.

51 Throughout acclimation and experimental periods, birds were weighed once weekly
52 to monitor body condition. Birds were held in the Ecological Research Centre (ERC) at the
53 University of Wollongong (34°25'S, 150°54'E). Upon introduction to the ERC, birds were
54 treated for internal and external parasites using Piperazine Solution (Piperazine anhydrous:
55 172 5g L⁻¹; Inca (Flight) Co. Pty Ltd, St Mary's, NSW, Australia) and Pestene-Insect Powder
56 (Sulfur: 50g kg⁻¹, Rotenone: 10g kg⁻¹; Inca (Flight) Co. Pty Ltd, St Mary's, NSW, Australia), and
57 birds were treated fortnightly henceforth. During the acclimation period, birds were housed
58 in groups for two weeks followed by a further ten days of acclimation to individual housing
59 conditions and adjust to the control and experimental diets (see Diet Composition and
60 Feeding Trials). Throughout the experimental period, birds were individually housed in
61 standardised stainless steel mesh cages (0.6 m wide x 0.6 m deep x 0.5 m high) equipped

62 with collection trays lined with waxed paper for excreta collection. Each cage was equipped
63 with plastic self-feeders for food and water; throughout the entire study food and water
64 were offered ad libitum. Birds were housed at temperatures between temperature of 22 –
65 25°C with air humidity 50 – 60% and a 14 h: 10 h light : dark regimen, using full-spectrum UV
66 fluorescent bulbs.

67

68 *Diet Composition and Feeding Trials*

69 Prior to the experimental period, birds were fed a standard commercial layer pellet diet
70 ('Back Yard Layer' - The Vella Group, Plumpton, NSW, Australia), containing 8.37% fibre as
71 fed (fibre type not defined). Animals were then assigned randomly to the experimental diet,
72 in this case a diet high in fibre (16% Neutral detergent fibre; see Courtney Jones et al. *In*
73 *press* for full details). Birds were acclimated to a high fibre experimental diet with a
74 transition period over 5-days that introduced the experimental diet incrementally (at 0%,
75 30%, 50%, 70%, 100%) to the low-fibre layer pellet diet. The control and experimental diets
76 were obtained from the Poultry Research Foundation of the University of Sydney, Camden,
77 New South Wales, Australia (see Table 1). Fibre for the experimental diets was sourced from
78 wheat bran. All diets were analysed to determine proximate chemical composition (Table 1).

79 *Body Mass, Feed Intake, Egg Production and Apparent Metabolisability*

80 Body mass, food intake and eggs laid were measured daily; all masses were measured in
81 grams, with the exception of body mass being measured in kilograms. Feed remaining and
82 spilt food were collected quantitatively and stored in a dry air tight room (see Sample
83 Analysis). After the 14 day experimental period, birds were euthanased to measure
84 morphometric differences.

85

86 The intake of dietary components (Dry Matter Intake) was calculated as [(Feed offered –
87 Feed Remaining and Spilt)*%Dry Matter]. The apparent and energy metabolisabilities (%)
88 of the dietary components were calculated as:

89 $(((\text{Dry Matter Intake} - \text{Dry Matter Output})/\text{Dry Matter Intake}) * 100$ and $(((\text{Dry Matter}$
90 $\text{Energy Intake} - \text{Dry Matter Energy Output})/\text{Dry Matter Energy Intake}) * 100$ respectively,
91 where Intake and Output were measured as g per chicken day⁻¹ (Robbins, 1993).

92 *Dissections and Morphometry*

93 Animals were euthanased using isoflurane overdose followed by cervical dislocation.
94 Immediately following euthanasia, macroscopic dissections and measurements of organ
95 size were conducted.

96 *Sample Analysis*

97 Daily samples of food offered, food remaining, food spilt and faecal output were collected
98 and weighed to determine wet mass. Faecal samples were stored frozen (-20°C). Faecal
99 samples from each day were then later thawed and sub samples (approximately 25% wet
100 mass) along with sub-samples of food offered were prepared (approximately 25% wet
101 mass) for analysis by air drying in an oven at 103°C for 48h (Robertson and Van Soest,
102 1981) to determine dry masses.

103 The dried samples of feed offered were ground using a Wiley Mill through a 1 mm
104 mesh (Arthur Thomas Co. Scientific Apparatus, Philadelphia, USA). Ash content of the
105 dried, ground feed offered were determined by dry ashing 0.5 g samples in a Thermolyne

106 Muffle Furnace (Model 62700; Dubuque, Iowa, USA) at 550°C for 12 h. Organic matter of
107 food stuffs was calculated as the Dry Matter content – Ash Content.

108 The Neutral Detergent Fibre (NDF; comprised of mainly cellulose, hemicellulose and lignin)
109 and Acid Detergent Fibre (ADF; comprised of mostly cellulose and lignin) of the three diets
110 offered were determined using reagents and procedures as described by Van Soest et al.
111 (1991); before neutral detergent digestion, duplicate 0.5 g sample bags were washed in
112 acetone to remove any soy bean products from the extract samples. Using a sequential
113 filter bag technique, to reduce any unintentional loss of samples during the procedure, and
114 ANKOM Fibre Analyser (Model 220, ANKOM Technology Corp., Fairport New York, USA),
115 the soluble cell contents (calculated as Dry Matter – Neutral Detergent Fibre),
116 hemicellulose (NDF – ADF) and cellulose contents (ADF – lignin) in the three diets were
117 determined by the difference. NDF and ADF were not adjusted for ash content and are
118 presented on a %DM basis only.

119 The energy content of the dried, ground feed and faeces were determined via the
120 combustion of duplicate ca. 0.5 g sub samples in a bomb calorimeter (Gallenkamp, Model
121 CB-375; Gallenkamp and Co. Ltd, Loughborough, UK); a standard of benzoic acid was used
122 for calibration. Nitrogen content of feed was determined by total combustion of duplicate
123 0.2 g samples in a Leco CHN-1000 elemental analyser (Leco Inc. St Joseph, Michigan, USA).

124

125 **Results**

126 We describe here an atypical caecal arrangement found in a hen that was otherwise
127 unremarkable with regard to body mass, digestive performance (apparent dry matter
128 metabolisability) and egg productivity (output and mass) when compared with other hens

129 fed similar diet. At evisceration the hen was 2.0 kg and was free from any other obvious
130 abnormalities. This is the first report of an abnormal caecal arrangement found in a mature,
131 otherwise healthy hen, and the first description of such an anomaly where digestive
132 performance and egg production were quantified.

133 Following euthanasia of the bird, examination of its gastrointestinal tract revealed an
134 atypical singular tubular outgrowth from the ileo-caecal junction, rather than the typical
135 paired outgrowths (Fig. 1). This singular tubular outgrowth, referred henceforth as the
136 caecal duct, bifurcated distally 60.3 mm from the ileo-caecal junction, and opened distally to
137 form two blind sacs, henceforth referred to as the paired caeca. These paired caeca shared
138 common mesentery with adipose tissue. Further, a small sacculation was present at the
139 junction of the caecal duct and the paired caeca (Fig. 1). Of the paired caeca, one was longer
140 and heavier (henceforth referred to as caecum 1) in comparison with the other (caecum 2).
141 Caecum 1 was 64.3 mm in length (from the point of bifurcation), and had diameters of 10.4
142 mm, 7.0 mm and 6.2 mm at the blind sac, mid-point (body) and proximal section
143 (immediately adjacent to the ileo-caecal junction), respectively (average diameter was 7.9
144 mm). Caecum 2 was 60.8 mm in length, and had diameters of 7.5 mm, 7.0 mm and 6.8 mm
145 at the blind sac, mid-point (body) and proximal section (immediately adjacent to the ileo-
146 caecal junction), respectively (average diameter 7.1 mm). Of note, the blind sac of caecum
147 1 arched and widened to bulge at the apex, but the caecum 2 blind sac was straight and
148 bluntly rounded at the blind end (Clench and Mathias, 1995). We also observed a twist in
149 the caecal duct immediately adjacent to the ileo-caecal junction.

150

151

152 **Discussion**

153 Similar caecal deviations have been described in adult chickens (e.g. Grewal *et al.*, 1976;
154 Peckham 1965), and in younger birds, including a 20-week White Leghorn pullet (Grewal *et*
155 *al.*, 1980) and in a 7-week old broiler (Mishra and Panda, 2010), but no indications of animal
156 performance were reported in these cases. Mishra and Panda (2010) noted that the paired
157 caeca of their broiler shared an extensive inter-caecal mesenteric fold and capillary bed;
158 however, this was not observed in our case study. Further, Grewal *et al.*, (1980) noted
159 complete aplasia of the left caecum, and on the right caecum a groove of the free surface
160 dividing the caecum into two separate structures terminating at the apex with two blind-
161 ended structures. In our case study, there was distinct singular proximal duct (tube), and
162 which possessed a short groove proximally on the free surface, but which did not extend
163 distally (Fig. 1). Furthermore, Grewal *et al.* (1976) noted that their 20-week old pullet with
164 an aplastic left caecum was lethargic and with a pale comb, but the bird was also infested
165 with jejunal round worms. We found no such malady in our mature hen.

166 Our hen was up to 18-months production (or 2 years old) and exhibited no
167 abnormalities of egg production or egg quality (egg number and mass) during our 28-day
168 feeding trial (Fig. 2). Furthermore, our hen showed no obvious deviations in feed intake,
169 apparent metabolisability of dry matter (%) or body mass changes when compared to all
170 other hens offered the same diet (a high-fibre mash, balanced for energy and protein,
171 matching contents a low-fibre, standard layer hen mash; Courtney Jones *et al.*, *In press*).
172 Thus, if the presence of an abnormal caecal arrangement reduced the digestive abilities of
173 our hen they were not obvious, and some compensatory mechanism/s may have existed.
174 Alternatively, the abnormal caecal arrangement of our hen, with its long singular duct and
175 short terminally paired caeca, might function adequately, such that no compensation in

176 feed intakes or reduced egg productivity was required (Fig. 2). Of note, Mishra and Panda
177 (2010) reported that the histological characteristics of an abnormal caecal duct were similar
178 to that of the ileum. Given that the ileum generally has a higher absorptive capacity for
179 various nutrients compared with the caeca, it is possible that similar morphological
180 characteristics allowed the abnormal caecum of our hen to compensate for lost nutrition
181 (Mishra and Panda 2010), but we were unable to conduct histological analyses in this case.

182 The single caecal duct seen here may be indicative of an error during organogenesis
183 during embryogenesis (Romanoff, 1960). Given similar deviations in caeca morphology have
184 been reported previously, this may indicate that errors in organogenesis may be more
185 frequent than previously thought in *Galliformes*, and in particular in domesticated species. It
186 is suspected that intensive breeding may result in such abnormalities, suggesting that
187 domestic chickens could be a useful model for investigating organogenesis of digestive
188 structures. Notably, the dimensions of our chicken's deviated caeca were approximately
189 double those observed in normal caecal arrangements for duct diameters of caecal material.
190 For example, the typical diameter of the caeca at the opening at the ileo-caecal duct for the
191 normal hens in our study were 7.6 ± 1.6 mm and 8.4 ± 1.8 mm for the right and left caecum,
192 respectively. In contrast, the diameter of the caeca at the opening at the ileo-caecal duct for
193 our chicken's deviant caeca was 11.6 mm. In addition, caecal-body diameters 60 mm distal
194 to the ileo-caecal duct in normal chickens were $5.5 \pm .07$ mm and 6.3 ± 1.9 mm for the right
195 and left caeca, respectively, compared with a diameter of 12.9 mm in the deviant caecum;
196 60 mm was the length at which the deviant caecum's common duct bifurcated (Fig. 1).
197 Furthermore, the entire caecal empty-wet mass of normal chickens fed the same diet was
198 7.7 ± 0.4 g, compared with 6.6 g for the entire empty wet-mass of the deviant caecum. As
199 such, the comparable dimensions of the deviant caecum relative to a normal paired caeca

200 may have compensated for the complete aplasia of one caeca, and may explain why no
201 deficits of health or production were noted in that chicken.

202

203 **References**

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219

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228 of Biology, Australian National University) for access to facilities and assistance with analysis of
229 samples. Sincere thanks to two anonymous referees whose comments and suggestions have
230 improved our manuscript.

231

232 **Figure Captions**

233 Figure 1: Aplasia of the left caeca; with only the right caeca present, bifurcation at the blind
234 end.

235

236 Figure 2: Comparison of mean body mass (g), body mass change (g), daily dry matter intake
237 (g d^{-1}), apparent dry matter metabolisability (%), total number of eggs and egg mass (g) of a
238 single layer hen with caecal abnormality (Individual), compared with the group means from
239 $n= 5$ hens fed the same diet, but with no gross caecal-abnormalities (High Fibre Balanced;
240 see Table 1 for details).

241

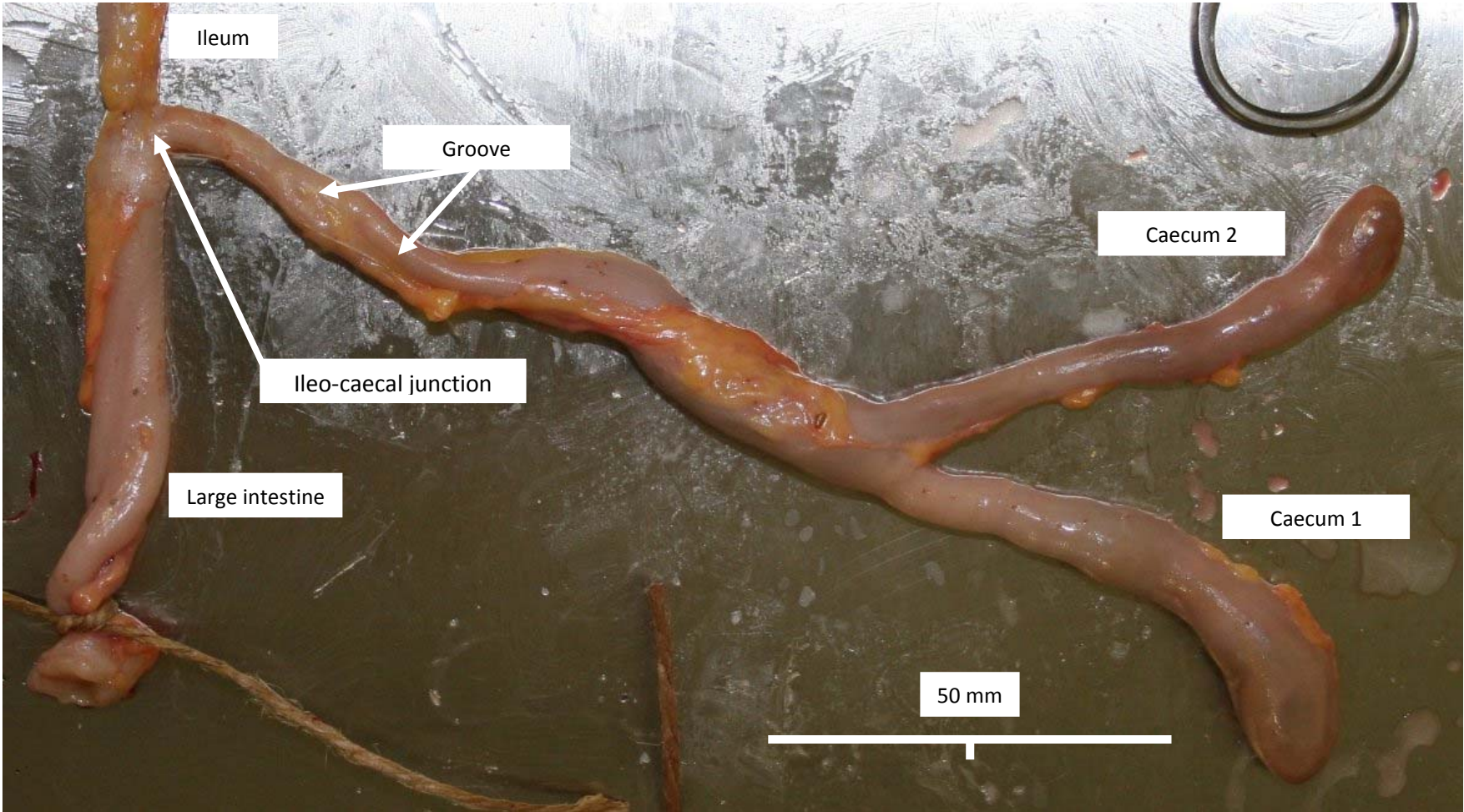
242

243 Table 1: Ingredients and proximate nutrient composition of the experimental diets used in this
 244 experiment; Low Fibre = standard layer mash, and High Fibre Balanced was diluted with wheat bran
 245 to increase fibre contents, and was balanced with corn oil to match the Low Fibre diet metabolisable
 246 energy contents.

247

% ingredients as fed	Low Fibre	High Fibre Balanced
Wheat – Feed	72.5	44.6
Soybean Meal 48	15.2	13.2
Wheat Bran	-	25.0
Corn Oil	2.1	7.1
Salt	0.14	0.17
Sodium Bicarbonate	0.21	0.17
DL Methionine	0.08	0.08
Lysine HCl	0.02	0.00
Limestone	8.8	8.8
Dicalcium Phosphate	0.77	0.77
Vitamin Premix	0.20	0.20
Chemical composition		
Dry Matter (%) [*]	92.1	92.0
Organic Matter (%) [*]	90.9 ± 0.1	86.7 ± 0.0
Nitrogen (%) [*]	3.1	3.0
Crude Protein (%) [*]	19.2	18.5
Gross Energy (kJg ⁻¹ DM as fed) [*]	16.4 ± 0.2	18.5 ± 0.8
Metabolisable Energy (kJd ⁻¹ DM)	11.5 ± 1.2	12.2 ± 0.7
Neutral Detergent Fibre (%) [*]	8.5±0.5	15.6 ± 0.5
Acid Detergent Fibre (%) [*]	3.0±0.01	5.9 ± 0.2

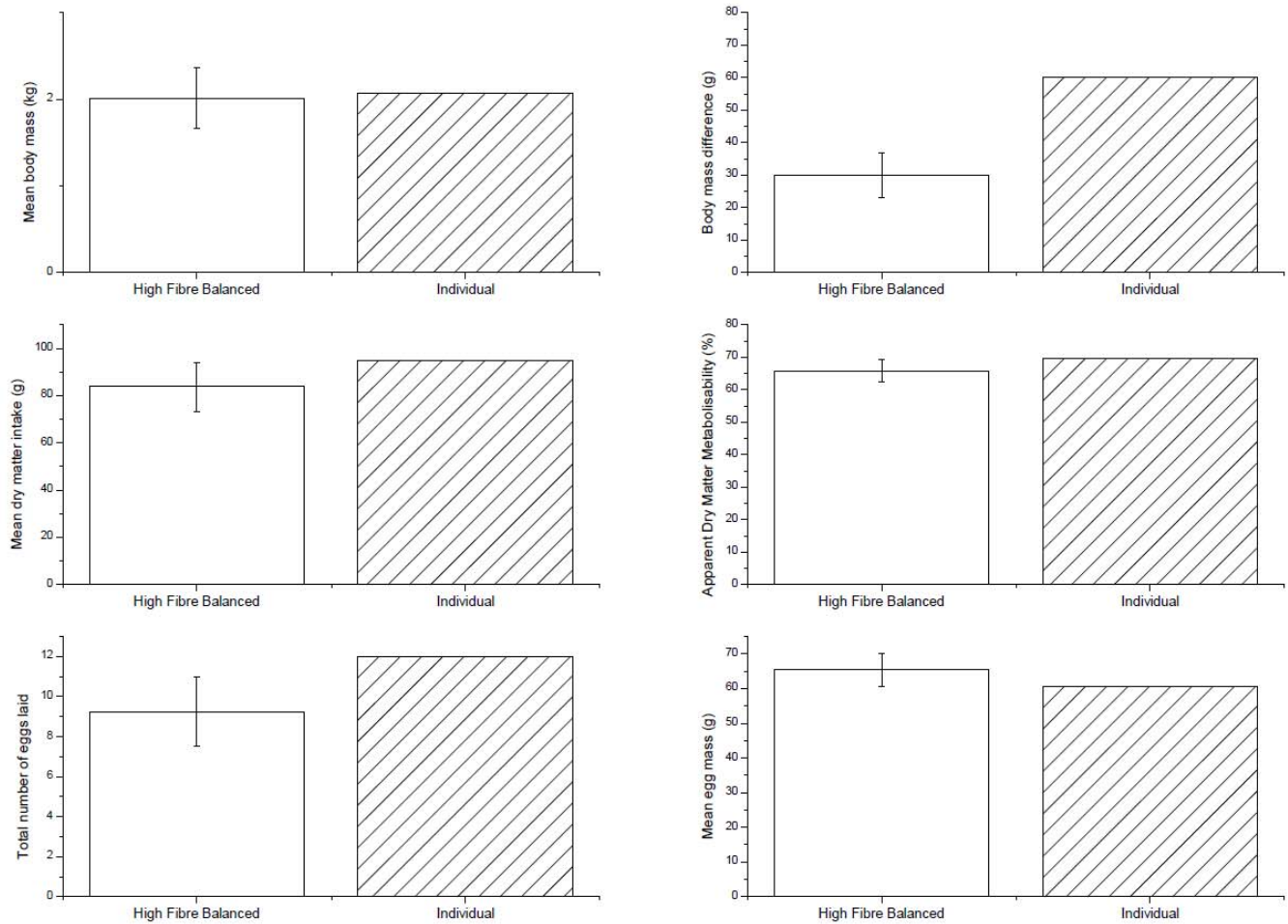
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249

250 Fig. 1

251



252 Fig. 2