Ultrasound evaluation of small intestinal thickness and a comparison to body weight in normal chickens (*Gallus gallus domesticus*)

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Background Ultrasound in avian patients is useful for identifying abnormalities within the coelomic cavity. A correlation between sonographic evaluation of jejunal thickness and body weight has been reported in mammals, but not the

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chicken (*Gallus gallus domesticus*). The purpose of this study was to prospectively assess the normal values of jejunal thickness in the chicken and compare this to body weight.

**Materials and methods** Coelomic ultrasound was performed on 89 clinically normal chickens with no history or signs of gastrointestinal disease. Two populations of hens (commercial layers and backyard purebred and mixed-breed hens) were used. Breed and ultrasonographically measured jejunal wall thickness were recorded in all hens. Body weight was recorded in 45 of the hens (mixed breed and purebred backyard chickens).

**Results and conclusion** There was no statistically significant correlation between body weight and ultrasonographically measured jejunal wall thickness. The mean thickness of the jejunal wall in healthy chickens was $2.1 \pm 0.08$ mm. Further studies comparing jejunal thickness in chickens with and without signs of GI disease would be useful.

**Key words** chickens; coelomic ultrasound; intestines; ultrasound

**Abbreviation** GI, gastrointestinal

Ultrasound is increasingly used as a diagnostic tool in veterinary practices to assess small intestinal thickness. In birds the presence of coelomic air sacs has been thought to inhibit evaluation of the coelomic cavity, as has the presence of
gas or ingesta in the gastrointestinal (GI) tract.\textsuperscript{1} However, more recent studies have demonstrated the usefulness of ultrasound in investigating GI disease in avian patients. For example, ultrasonographic evaluation of duodenal thickness in pigeons demonstrated a statistically significant difference in the thickness of the intestinal wall between healthy pigeons and pigeons with GI disease.\textsuperscript{2} In Australia, chickens have been identified as the third most popular species of pet bird,\textsuperscript{3} but the normal and abnormal ultrasonographic findings of the GI tract have been rarely reported.\textsuperscript{4} Ultrasound in chickens has the potential to aid in the investigation of GI disease such as coccidiosis, colibacillosis and necrotic enteritis, which may alter intestinal wall thickness. The focus of the present study was to evaluate the range of normal jejunal thickness on ultrasound in chickens without clinical signs of GI disease, using a range of breeds of domestic chickens (\textit{Gallus gallus domesticus}: commercial layers and backyard hens). Additionally, a comparison between intestinal thickness and body weight was performed in the backyard hens.

\textbf{Materials and methods}

Healthy hens without clinical or historic evidence of GI disease or other known illnesses were enrolled in this prospective study, with data collected over a 12-month period. Hens with a history of any illness (e.g. being scanned as part of a
clinical investigation for suspected illness or hens with coelomic abnormalities identified on ultrasound) were excluded. Hens were either commercial layers (n = 44, of which 36 were part of a university-owned research flock and 8 were rehomed chickens) or backyard hens owned by staff, students or clients of the University Veterinary Medical Centre (n = 45). Of the backyard hens, purebred chickens, including ISA Brown, Leghorn, Araucana, Maeraucana, Australorp and Wyandotte, and mixed-breed chickens were represented. The project was approved by the University of Queensland Animal Ethics Committee (Approval no. SVS/150/15).

Fasting or sedation was not performed prior to ultrasound. A physical examination was performed immediately prior to ultrasound by a clinician (KG or BD). The hens were not clipped or plucked; the feathers were parted for the ultrasound with acoustic coupling gel applied to the apterylae between the feathered pterylae. The hens were manually restrained either standing or in dorsal recumbency and scanned by a single author (KG, see Figure 1). Scans were performed on one of two ultrasound systems (Philips EPIQ 5 or Mindray Z6 ultrasound machines) using an 8-MHz curvilinear transducer. The coelomic cavity was examined in a fanning motion from a left lateral approach and a segment of jejunum identified. Measurements of the jejunum were made at the
time of scanning on static ultrasound images with the intestinal segments in long-axis orientation (Figure 2). Measurements were performed once the intestinal wall layers were clearly visualised and in most birds a single measurement was recorded. If multiple measurements were recorded, the measurement made on the best quality image was used. Measurements were made from the mucosal to serosal layers, using electronic callipers positioned in a leading-edge-to-trailing-edge fashion. In all hens, breed and ultrasonographic jejunal wall thickness was recorded. In the 45 backyard hens, body weight was also recorded.

**Statistical analysis**

Descriptive summary statistics for ultrasonographic jejunal thickness in centimetres and body weight in kilograms were recorded, and visualised using box plots and dot plots. Normality of data was tested for using the Shapiro-Wilks W test, with Grubbs and generalised extreme studentised deviate (ESD) tests to test weight data for the presence of outliers. Mean jejunal thickness for commercial layer and backyard hens was compared using a Student’s t-test. For backyard hens, an association between body weight and jejunal thickness was investigated using linear regression. A quadratic weight term was added to the model to test for evidence of a non-linear relationship and was removed if non-
significant. For all appropriate statistical tests, significance level was set at $\alpha = 0.05$.

All statistical analyses were conducted in Stata (version 14) and conducted by one author (NP).

**Results**

The chickens were classified into two general groups: commercial layer hens ($n = 44$) and backyard hens ($n = 45$ comprised of purebred ($n = 13$) and mixed breed ($n = 32$) hens). An additional 4 hens were scanned and subsequently removed from the study because of ultrasonographically identified coelomic abnormalities. No abnormalities were identified on physical examination of the study birds. Several breeds and mixed breeds were represented, with body weights ranging from 1 to 3.27 kg. One hen (4 years old, Sussex hen weighing 4.65 kg) was removed from the analysis because the body weight for this hen was classified as an outlier on the Grubbs and generalised ESD tests when compared with all other body weight data. A combination scatter plot (Figure 3) showed normal distribution of the data for jejunal thickness and the Shapiro-Wilk $W$ test of normality indicated the jejunal thickness data were normally distributed ($P = 0.16$). There was no difference in mean jejunal thickness
between the commercial layers and backyard hens. The range of jejunal thickness was 1.2–3.1 mm.

In all birds, a segment of jejunum could be visualised and measured. Hyperechoic intestinal content was frequently identified. In all birds, the jejunal layers were identified and were distinctly visible; the mucosa and muscularis were hypoechoic and the submucosa and serosa were hyperechoic. Descriptive statistics for sonographically measured jejunal thickness are provided in Table 1. The mean jejunal thickness was 0.216 ± 0.08 cm.

In the backyard hen group, linear regression analysis indicated there was no statistically significant relationship between jejunal thickness and body weight (P = 0.27; Figure 4). Addition of a quadratic weight term to the regression model did not improve model fit (P 0= 0.99) and this term was removed from the final model.

Discussion

This preliminary prospective study assessed normal values for jejunal thickness in healthy chickens using ultrasound. The left lateral approach was used in all chickens to ensure the duodenum (which is normally located on the right side of the coelomic cavity) was not inadvertently selected. Avian patients are increasingly being presented for evaluation in veterinary hospitals worldwide.
and coelomic ultrasound is a useful tool in the investigation of many coelomic diseases,\textsuperscript{2,4–7} including hepatomegaly, GI disease and reproductive diseases such as salpingitis and egg yolk peritonitis.\textsuperscript{3,5,6} Ultrasonography of the normal avian GI tract has been described in 12 pigeons\textsuperscript{5} and a statistically significant difference was found in the duodenal thickness between healthy pigeons and those with GI disease. Thus, it is expected that similar changes in intestinal thickness would occur in chickens with GI disease. Further studies comparing jejunal thickness in chickens with and without signs of GI disease would be useful.

\textit{Study limitations}

We only measured jejunal segments alone and not the duodenum, which may be slightly thicker than the jejunum in other species.\textsuperscript{7,8} For example, in dogs the normal duodenal thickness is 3–6 mm, while the jejunum is 2–5 mm thick.\textsuperscript{9} In cats, the reference ranges are the same for the duodenum and jejunum (2.0–2.5 mm). The relevance is that additional studies could be performed to establish if duodenal thickness is different to jejunal thickness in chickens. The duodenum was not examined in this study because of the difficulty in consistently identifying it.
Despite obtaining a clinical history of normality, four of the hens examined were found to have a coelomic effusion (some with concurrent reproductive abnormalities) identified incidentally on ultrasound. These hens were subsequently excluded from analysis and did not form part of the described study group. Therefore, it is possible that other hens in the groups examined had asymptomatic illnesses that were not identified on clinical history or ultrasound examination as no further investigations (e.g. necropsy or histopathology) were performed. Given the minimally invasive study design and pet status of most hens, it was not feasible to have normal necropsy findings as an inclusion criteria.

Use of a higher frequency transducer (e.g. 18 MHz) rather than an 8-MHz transducer would have improved the resolution of the ultrasound images. For the purpose of this study, an available higher frequency transducer had a larger linear coupling surface, which had poor skin contact because of the dense feathering of the birds in the study. This impeded image quality, so was not used.

In conclusion, this study did not show a correlation between body weight and jejunal thickness in chickens. In all chickens, the wall layering was distinct and
easily visualised. The mean jejunal wall thickness in healthy chickens was 2.16 ± 0.08 mm and the range was 1.2–3.1 mm.

Acknowledgment

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Conflicts of interest and sources of funding

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References


Figure 1. Photograph demonstrating the left lateral approach to ultrasound examination of the coelomic cavity of a chicken.

Figure 2. Static image of a jejunal segment of a chicken in long-axis orientation. The callipers indicate the intestinal wall measurement from the edge of the
intestinal lumen (which contains a small amount of ingesta) to the edge of the serosa.

Figure 3. Scatter plot of jejunal thickness (cm) in commercial layer (n = 44) and backyard hens (n = 45). The mean and SD are indicated by the central line and whiskers. There was no significant difference in mean jejunal thickness (P = 0.14).

Figure 4. Scatter plot of jejunal thickness (cm) against body weight (kg) in 44 backyard hens. The straight line is a fitted regression line and the shaded area shows the 95% confidence interval for the fitted line across the observed range of weight values. The regression equation is shown at the bottom of the plot. There is no significant association between jejunal thickness and body weight (P = 0.27).
Table 1. Descriptive statistics for jejunal thickness for each of two categories of hens

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Mean (cm)</th>
<th>SE</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial layers</td>
<td>44</td>
<td>0.222</td>
<td>0.005</td>
<td>0.213</td>
<td>0.231</td>
</tr>
<tr>
<td>Backyard hens</td>
<td>45</td>
<td>0.210</td>
<td>0.006</td>
<td>0.198</td>
<td>0.223</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>0.216</td>
<td>0.004</td>
<td>0.208</td>
<td>0.224</td>
</tr>
</tbody>
</table>

CI, confidence interval for the mean; SE, standard error.
jejunal thickness = 0.182 + 0.0136*weight
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