INTRODUCTION

In practical poultry nutrition, ME is standard and is calculated by subtracting fecal, urinary, and gaseous energies from gross energy. Energy models for laying hens typically account for ME partitioned to maintenance, growth, and egg production (Sakomura, 2004). These models allow for precise matching of nutrients supplied in the diet with nutrient requirements (Sakomura et al., 2011). Approximately 84% of the retained energy in eggs is contained in the yolk, yet there is scarce information about the partitioning of ME for ovarian follicle growth.

Avian follicular development is a continuous process from the activation of small cortical follicles to the ovulation of hierarchical follicles. Small white follicles are recruited into a hierarchy of large preovulatory follicles and become yellow due to yolk deposition. The largest follicle (F1) ovulates on an almost daily basis and is replaced by a new prehierarchical follicle that is selected to enter the hierarchy each day (Robinson et al., 2003; Diaz and Anthony, 2013). Large yellow follicle growth begins when a small yellow follicle is recruited into the rapid growth phase (RGP). During this phase, the follicle undergoes growth by deposition of yolk to the follicle periphery for 7 to 11 d (Gilbert, 1971). The follicle then ruptures and the ovum is released into the oviduct where albumen, membranes, and shell are deposited before oviposition.

At any one time, there are multiple follicles at different stages of maturity, typically weighing from 0.5 to 19 g (Gilbert, 1971). Development can be seen by the use of a double-dye technique, which involves feeding 2 different colored fat-soluble dyes, such as Scarlet Red and Sudan Black, on alternating days to the hens (Bruggeman et al., 2005). The yolk contains rings of
red and black dye, respectively, and the volume of yolk deposited each day can be estimated using radial measurements of the rings and the formula for the volume of a sphere.

Our hypothesis was that the modern laying hen partitions a greater amount of energy toward reproduction than a heritage line laying hen. The objectives of the current experiment were to estimate the rate of yolk deposition in a commercial line and a heritage line of laying hens, and to develop a predictive mathematical model of follicle growth.

**MATERIALS AND METHODS**

**Bird Use**

The animal protocol for the study followed principles established by the Canadian Council on Animal Care Guidelines and Policies (CCAC, 1993), and was approved by the University of Alberta Animal Care and Use Committee for Livestock.

**Experimental Design**

Two strains of Single Comb White Leghorn (SCWL) hens were chosen: the H&N Nick Chick strain (Cuxhaven, Germany) was chosen as the modern commercial line (CL), and a strain obtained by the University of Alberta in 1992 from Roy Crawford (University of Saskatchewan) that was unselected since 1967 was chosen as the heritage line (HL). Eight SCWL hens were randomly assigned to each of 3 treatments, in a completely randomized design: 1) HL hens and 2) CL hens, both fed dye capsules containing Sudan IV and Sudan Black dyes on alternate days; and 3) a control treatment where CL hens were fed empty capsules daily. The control treatment (CL hens only) was used to determine the effect of dye feeding on egg production and

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Rings of alternating color resulting from the use of a double-dye technique in Single Comb White Leghorn hens of a commercial and an unselected heritage line. The radius of each concentric ring was measured in duplicate (perpendicular directions) and used to determine the volume of yolk deposited daily. Color version available in the online PDF.
feed intake, with the assumption that if the dye had no effect on CL performance, then it would also have no effect in HL hens. An individual hen was considered an experimental unit.

**Stock and Management**

A total of twenty-four 32-wk-old SCWL hens were used in this study. The birds were randomly allocated in single-bird cages and housed in an environmentally controlled poultry research facility with a photoperiod of 15L:9D and an ambient temperature of 22°C. All birds were fed a standard phase 1 layer diet (2,800 kcal/kg of ME; 18.5% CP) ad libitum. Body weight and feed intake was measured daily. On d 22 of the trial, all birds were euthanized by cervical dislocation and carefully dissected to evaluate ovary morphology. Ovaries were examined for signs of follicular atresia and any abnormalities. Follicles greater than 1 cm in diameter where removed from ovaries, individually boiled, and weighed.

**Dye Feeding**

A double dye technique adapted from similar experiments conducted by Bacon and Skala (1968), Gilbert (1972), Yu et al. (1992), Bruggeman et al. (2005), and Qin et al. (2013) was used to measure daily yolk de-

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**Table 1. Energy content of each fraction of yolk, albumen, and shell components of a chicken egg**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yolk kcal/g</th>
<th>%</th>
<th>kcal/g</th>
<th>Albumen kcal/g</th>
<th>%</th>
<th>kcal/g</th>
<th>Shell kcal/g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0</td>
<td>56.92</td>
<td>0</td>
<td>0</td>
<td>87.92</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4.0</td>
<td>3.03</td>
<td>0.12</td>
<td>0.28</td>
<td>0.09</td>
<td>0.00034</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lipids</td>
<td>9.2</td>
<td>25.42</td>
<td>2.34</td>
<td>0.09</td>
<td>0.09</td>
<td>0.0021</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protein</td>
<td>4.1</td>
<td>13.44</td>
<td>0.55</td>
<td>11.07</td>
<td>2.01</td>
<td>0.08</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Inorganic material</td>
<td>0</td>
<td>1.19</td>
<td>0</td>
<td>0.64</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total kcal/g 3.01 0.47 0.08

1 Data from Radu-Rusu et al. (2012) and Gilbert (1971).

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Figure 2. Rapid growth phase (RGP) ovarian follicle weights predicted by the Lomolino model after recruitment into the RGP. The overall mean is represented by the solid line, and strain variation is shown by dashed lines. Data are from Single Comb White Leghorn hens of a commercial and an unselected heritage line.
position. For 21 consecutive days, the birds were fed a capsule (no. 1) containing dye between 0730 and 0800 h. Eight HL and 8 CL hens were fed capsules containing either 15 ± 2 mg of Sudan IV (scarlet) dye or 20 ± 2 mg of Sudan Black on alternating days. The control hens were fed an empty capsule. Capsules were deposited in the back of the mouth by hand, and swallowed by the birds.

Daily, eggs were collected, labeled, weighed, and boiled for 15 min. Once boiled, eggs were transported to a laboratory where they were processed within 4 d of collection. Dye ring analysis excluded any eggs before d 10 of the trial to eliminate eggs that entered the RGP before the start of feeding dye capsules.

**Yolk Deposition Rate Estimation**

Ova were isolated and weighed before being bisected using a sharp heavy-duty utility knife. The center of each ovum was marked with a pin and the radius of each dye ring was measured. Using the computer program AxioVision LE (Zeiss, Oberkochen, Germany), high definition photos were taken of the dye rings. Duplicate perpendicular dye ring radii were measured from the center of the yolk to the inner edge of each ring (Figure 1), and the volume of yolk deposited was estimated using the formula for the volume of a sphere:

\[ V = \frac{4}{3} \pi r^3, \]

where \( V \) is the volume and \( r \) is the radius. The volume of the yolk deposited daily was calculated as the difference in total yolk volume between 2 consecutive rings.

**Estimating Energy Cost for Egg Production**

For comparison, overall energy partitioning coefficients were estimated empirically using the following model (adapted from Sakomura, 2004):

\[ \text{ADMEI} = a \times \text{BW}^{0.67} + s \times \text{BW}^{0.67} + g \times \text{BWG} + e \times \text{EM}, \]

where ADMEI is the average daily ME intake (kcal); BW is in kilograms; BWG is the BW gain (g/d); EM is the egg mass; \( a, g, \) and \( e \) are coefficients for maintenance, growth, and egg production energy requirements, respectively; \( s \) is a coefficient for strain differ-

![Figure 3. Rapid growth phase (RGP) ovarian follicle weights predicted by the Lomolino model. Data are from Single Comb White Leghorn hens of a commercial and an unselected heritage line. The overall mean is represented by the solid line. Curves connected by dashed lines represent follicles maturing (ovulated) after 6, 7, 8, or 9 d in the RGP.](image-url)
ences in maintenance ME requirement (where strain is CL, $s = 0$; where strain is HL, $s = 1$).

The method used to estimate the energy content of egg components (yolk, albumen, and shell) was summarized in Table 1. The density of yolk was estimated at 1.0 g/cm$^3$ (Sousa et al., 2007); DM was 43.1%; and GE retained in whole yolk was 3.01 kcal/g (Radu-Rusu et al., 2012).

The nonlinear Lomolino growth model (Faridi et al., 2014) was used to describe follicle growth during the RGP for both strains:

$$ W_t = \frac{W_m}{1 + b \ln\left(\frac{t}{t_0}\right)} $$

where $W_t$ is the follicle weight (g) at time $t$ (d), $W_m$ is the asymptotic follicle weight (g), $b$ is a coefficient of follicle maturation rate, $k$ is a constant, and $t$ is time since the start of the RGP (d). Analysis of the residuals using a Gompertz model revealed a pattern relative to the time follicles spent in the RGP, indicating that the predicted values from the Gompertz model were biased (data not shown). Residuals from the Lomolino model, on the other hand, were randomly distributed with respect to time in the RGP. Thus, the Lomolino model was chosen as a more appropriate model to explain follicular weight development during the RGP.

### Statistical Analysis

The NLIN procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) was used to determine energy partitioning coefficients, and coefficients for the Lomolino model. Residual values of the Lomolino model were tested for significant treatment differences using the MIXED procedure of SAS. One-way analyses of variance were conducted using the MIXED procedure of SAS to determine differences in dependent variables (BW, feed intake, and egg weight) due to strain. The effect of feeding dye versus the empty capsules (control treatment) on performance parameters was conducted independently because the control birds were only in the CL line. Differences between treatments were reported at a significance level of $P < 0.05$.

### RESULTS AND DISCUSSION

#### Live Performance

During the experiment, the average BW for CL and HL were 1.637 and 1.596 kg, respectively. Hen-day egg production was higher in CL than in HL: 95 and 68%, respectively. The CL strain had an average egg weight of 61.4 g, and HL had an average egg weight of 56.4 g. The CL hens had 42 kcal/d higher ME intake (312 kcal/d) compared with the HL hens (270 kcal/d), and

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Figure 4. Increase in ovarian follicle radius during the rapid growth phase (RGP). Overall pattern for all follicles from a commercial and an unselected heritage line of Single Comb White Leghorn hens. Points with no common letter (a–f) differ significantly ($P \leq 0.05$).
Figure 5. Effects of strain (panel A) and total number of days in rapid growth phase (RGP) before ovulation on the increase in ovarian follicle radius. Points within day of development with no common letters (a–c) differ significantly ($P < 0.05$).
a higher empirical estimate of ME partitioned to maintenance (191.6 vs. 176.6 kcal/kg₀.⁶⁷, respectively). The coefficients, \( a \), \( g \), \( e \), \( s \), and \( b \), for the energy partitioning model were 191.6, 0.56, 0.74, −14.95, and 0.68, respectively, resulting in the following equation:

\[
ADMEI = 191.6 \times BW^{0.68} - 14.95 \times BW^{0.68} \\
\times \text{strain} + 0.56 \times BWG + 0.74 \times EM.
\]

In the current study, the energy required for maintenance of a larger body, increased egg production rate, and higher egg weight of the CL hens all contributed to higher feed intake. This in turn resulted in higher heat production, which manifested as a higher maintenance ME requirement.

The higher maintenance requirement (\( \text{ME}_{\text{m}} \)) in the CL hens was likely due at least in part to diet-induced thermogenesis. Romero et al. (2009), in describing residual maintenance requirement (\( \text{RM}_{\text{ME}} \)), demonstrated that the maintenance requirement in broiler breeders increased with higher feed intake. Their work predicted that diet-induced heat production would increase the maintenance requirement by 0.34 kcal per kg₀.⁵⁴. This prediction compares favorably to the empirically estimated increase in ME in the CL hens, which was 14.95 kcal/kg₀.⁶⁷ in the current study.

In the current study, the energy coefficients for BW gain (0.56 kcal/g) and for egg mass (0.74 kcal/g) were low relative to the retained energy in body tissue and eggs. Variation in energy partitioning coefficients is a common issue when using empirical estimation methods, especially with relatively small data sets, such as in the current study. Direct estimation of energy requirements using a follicle growth model is therefore advantageous.

### Follicular Rapid Growth Phase

Coefficients for the Lomolino model were \( W_m = 20.84 \text{ g}, b = 16.55 \), and \( k = 6.018 \). Thus, the following equation predicted follicular growth as a function of time (\( t \); days) in the RGP:

\[
W_t = \frac{20.842}{1 + 16.55 \ln \left( \frac{0.018}{t} \right)}.
\]

The derivative of the Lomolino equation can be used to calculate the growth rate of follicles over time (\( \delta W_t \)):

\[
\delta W_t = \frac{W_m \times \ln(b) \times b^{\ln(k)/t}}{t \times b^{\ln(b)/t - 1}^2}.
\]

### Table 2. Daily volume of yolk deposited in a single large yellow follicle by Single Comb White Leghorn hens of a commercial and an unselected heritage line

<table>
<thead>
<tr>
<th>Time before ovulation (d)</th>
<th>Commercial</th>
<th>SEM</th>
<th>Heritage</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.513⁵</td>
<td>0.055</td>
<td>1.925⁵</td>
<td>0.069</td>
</tr>
<tr>
<td>−1</td>
<td>2.860⁶</td>
<td>0.046</td>
<td>2.318⁶</td>
<td>0.058</td>
</tr>
<tr>
<td>−2</td>
<td>2.832⁷</td>
<td>0.042</td>
<td>2.479⁷</td>
<td>0.053</td>
</tr>
<tr>
<td>−3</td>
<td>2.479⁸</td>
<td>0.041</td>
<td>2.490⁸</td>
<td>0.052</td>
</tr>
<tr>
<td>−4</td>
<td>1.836⁹</td>
<td>0.052</td>
<td>2.146⁹</td>
<td>0.066</td>
</tr>
<tr>
<td>−5</td>
<td>0.985⁹</td>
<td>0.051</td>
<td>1.397⁹</td>
<td>0.066</td>
</tr>
<tr>
<td>−6</td>
<td>0.431¹</td>
<td>0.042</td>
<td>0.715¹</td>
<td>0.052</td>
</tr>
<tr>
<td>−7</td>
<td>0.275¹</td>
<td>0.045</td>
<td>0.329¹</td>
<td>0.040</td>
</tr>
<tr>
<td>−8</td>
<td>0.170¹</td>
<td>0.098</td>
<td>0.168¹</td>
<td>0.076</td>
</tr>
</tbody>
</table>

¹Means with no common superscript differ significantly (\( P < 0.05 \)).

²Number of days in the rapid growth phase before ovulation.

Analysis of the residuals from the Lomolino model showed a strain effect on time in the RGP (Figure 2). Follicles from the CL hens grew faster, such that follicles of CL hens were significantly heavier than follicles of HL hens on d 6 and 7 of the RGP. However, CL hens ovulated their follicles earlier than HL hens (7.35 vs. 7.95 d, respectively; \( P < 0.001 \)). This RGP duration was consistent with average RGP lengths of 7 to 11 d reported by previous researchers (Bacon and Skala, 1968; Grau, 1976; William and Sharp, 1978; Gilbert et al., 1983; Yu et al., 1992; Bruggeman et al., 2005). The average duration of RGP in the current study was shorter than that reported by William and Sharp (1978), Gilbert et al. (1983), and Bruggeman et al. (2005) in hens at older ages. William and Sharp (1978) and Alvarez and Hocking (2012) proposed that as laying hens aged there was a decrease in egg laying rate due to a reduction in rate of recruitment of yellow follicles and an increase in time for the follicles to reach the condition necessary for ovulation, thus increasing the duration of RGP. Another contributing factor could be atresia of large yellow follicles, which increases with hen age and contributes to a lower number of successful ovulations (Gilbert et al., 1983; Hocking et al., 1987). We did not observe any follicular atresia in the current study (data not shown).

There were no significant differences between the final follicle (ovulated ovum) weights 14.55 g (CL) vs. 14.22 g (HL), likely because the HL follicles had a slower rate of development and were subsequently in the RGP for a longer period of time. William and Sharp (1978) found an age-related difference in average F1 follicle weight (the next follicle to ovulate) in laying hens at 26, 52, and 113 wk of age (12.7, 15.7, and 16.3 g, respectively). Nonlinear interpolation of those values confirms that the 32-wk ovum weights in the current study were in an expected range.

The length of time follicles spent in the RGP (deduced from the number of dye rings in the ovum) was negatively correlated to the rate of follicle growth (Figure 3). Analysis of residuals from the Lomolino model showed that follicular weights for d 2 and higher of the
RGP were higher for follicles ovulating on d 6, which were higher than for follicles ovulated on d 7, which were higher than for those ovulated on d 8, which in turn were higher than for those follicles ovulated on d 9 of the RGP.

Regardless of the time in RGP, there were no significant differences in ovulated ovum weights with an overall average of 14.3 g. Bacon and Skala (1968) also reported no differences in final follicular weights during the RGP of follicles with varying number of dye rings over time. Significant differences in follicle weights using the dye ring technique over time in RGP in laying hens were observed in previous work by Lacassagne (1960) and Zakaria et al. (1984) who attributed this variation to the position of the egg within a clutch (sequence) and length of clutch, which was not observed in the current study (data not shown).

**Follicle Radius and Energy Retention**

Deposition of yolk material caused the radius to increase by 2.79 mm/d during the first 2 d of the RGP. After d 2, there was a linear decrease in thickness of yolk deposited that reached 0.61 mm/d by d 9 of the RGP (Figure 4). Our results were consistent with those reported by Bruggeman et al. (2005), who found a linear decrease in thickness of daily yolk deposition in dwarf breeder hens during the RGP. There was no difference in follicle radial growth due to strain or length of time in RGP after the second day of the RGP. However, radial growth was greater in the first 2 d of the RGP in the HL hens and in follicles that spent less total time in the RGP (Figure 5).

For some modeling exercises, it can be beneficial to work backward from the time of ovulation. The daily volume of yolk deposited during the RGP (Table 2), and gross energy partitioned for follicle development \( (E_{\text{retained}}) \), was a simple linear function of yolk volume \( (E_{\text{retained}} = \Delta V \times \rho \times E_y) \) Figure 6, where \( \Delta V \times \rho \times E_y \) was the increase in the volume of the follicle \( (\text{cm}^3/d) \); \( \rho \) was the density of yolk \( (1 \text{ g/cm}^3) \); and \( E_y \) was the energy content of whole yolk \( (3.01 \text{ kcal/g; Table 1}) \). Due primarily to differences in hen-day production, CL hens partitioned a greater amount of energy than HL line hens toward follicular development (13.64 vs. 9.48 kcal/d, respectively). Working backward from the time of ovulation, HL hens had a higher early rate of yolk deposition 7 to 5 d before ovulation, whereas the CL hens had a higher rate of yolk deposition 3 to 1 d before ovulation with no significant difference between lines on d 4 before ovulation. The HL hens invested more energy earlier in the RGP than CL hens, and therefore had an elongated growth curve compared with CL hens (Figure 6). This may be a result of selection for early onset of lay, as birds that come into lay earlier have a shorter RGP and a steeper growth curve (Gilbert et al., 1983).

The current study found that the Lomolino model could be used to explain follicular weight gain during the RGP in an unbiased manner. The CL hen ovarian

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**Figure 6.** Energy deposited in ovarian large yellow follicles in the rapid growth phase of Single Comb White Leghorn hens of a commercial and an unselected heritage line. Points with no common letter (a–j) differ significantly \( (P \leq 0.05) \).
follicles grew faster and ovulated earlier than in the HL hens, spending less time in the RGP. Daily increases in follicle radius decreased in magnitude over time during RGP in a linear manner. Considerable variation in RGP duration was observed. Faster growing follicles were ovulated after a shorter time in the RGP, suggesting conservation of the size of follicle at the time of ovulation. On average, commercial hens partitioned a greater amount of energy toward follicular growth daily than heritage hens.

REFERENCES


