The Riboflavin Requirement of Adult Dogs at Maintenance Is Greater than Previous Estimates\textsuperscript{1,2,3}

\textbf{JILL L. CLINE, JACK ODLE AND ROBERT A. EASTER\textsuperscript{4}}

Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801

\begin{abstract}
A study was conducted to determine the riboflavin requirement of adult dogs at maintenance. Twenty adult mixed breed dogs were fed a semipurified meal with one of five riboflavin concentrations: Diet 1, 1.7 mg/kg; Diet 2, 2.7 mg/kg; Diet 3, 3.7 mg/kg; Diet 4, 4.7 mg/kg; and Diet 5, 5.7 mg/kg. The erythrocyte glutathione reductase activity coefficient (EGRAC) was used to determine biochemical riboflavin deficiency. Dogs fed Diet 1 had a greater ($P < 0.05$) EGRAC (1.24) on d 56 of the trial compared with that of dogs fed Diet 5 (1.11), indicating marginal riboflavin deficiency in dogs fed Diet 1. On d 84 the mean EGRAC for dogs fed Diet 1 (1.36) was different from EGRAC obtained for dogs fed the other diets (1.19, $P < 0.05$). This difference in mean EGRAC was still present on d 112 (1.59 vs. 1.27; $P < 0.01$). There was no difference in d 112 mean EGRAC for dogs fed Diets 2, 3, 4 and 5 ($P < 0.05$). The broken line requirement estimate for the adult dog at maintenance was determined to be 66.8 \text{ mg riboflavin} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1} using the d 112 EGRAC as the basis for assessing biochemical riboflavin deficiency. J. Nutr. 126: 984–988, 1996.
\end{abstract}

\textbf{INDEXING KEY WORDS:}
\begin{itemize}
\item riboflavin
\item dogs
\item requirements
\end{itemize}

Cereal grains are a common ingredient in many commercial pet foods. Most grains are a poor source of riboflavin. There is little research available on which to base a riboflavin requirement for mature dogs. Most of the research has addressed the riboflavin requirement of young growing dogs. The adult requirement has been extrapolated from these values and is currently accepted as 50 \text{ mg} \cdot \text{kg body wt}^{-1}.

Riboflavin-deficient dogs display flaky dermatitis, muscle weakness, erythema, ataxia and ocular lesions. In addition, dogs also exhibit a unique phenomena, the collapse syndrome (Street and Cowgill 1939). Street and Cowgill (1939) used the collapse syndrome as an indicator of deficiency. They reported loss of appetite and muscular weakness before collapse. These investigators found that 25 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1} prevented collapse in adult dogs (Street and Cowgill 1939). In a further study, Street et al. (1941) reported degeneration of the myelin sheath of the posterior spinal cord and the peripheral nerves when adult dogs were fed 8 \text{ mg} of riboflavin per day for > 400 d. They suggested this as the basis for the incoordination and a loss of deep muscle reflex in the hind limbs observed in the deficient dogs.

Axelrod et al. (1941) used urinary riboflavin excretion levels to determine the requirement of puppies. From their results, a riboflavin requirement of 100–200 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1} was suggested for young growing dogs. Potter et al. (1942), using the same indicators, reported that the riboflavin requirement for puppies was between 60 and 100 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}. From this observation, Potter et al. (1942) extrapolated that the requirement for adult dogs was between 30 and 50 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}.

The requirement established by Axelrod et al. (1941) and Potter et al. (1942) was accepted at the time; however, urinary riboflavin excretion reflects dietary riboflavin rather than body flavin stores. In addition, urinary riboflavin is influenced by age, stress, sleep, nitrogen balance, physical activity and boric acid intake (Cooperman and Lopez 1984). Thus, more work was

\textsuperscript{1} Part of a thesis submitted to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the M.S. degree in Animal Science.

\textsuperscript{2} Presented as an abstract at the XVIII World Congress of the World Small Animal Veterinary Association. [Cline, J. L. & Easter, R. A. (1993) Riboflavin requirement of the adult dog at maintenance. XVIII World Congress WSAVA Proceedings, p. 380.]

\textsuperscript{3} The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

\textsuperscript{4} To whom reprint requests should be addressed: 208 Animal Sciences Laboratory, 1207 W. Gregory Drive, Urbana, IL 61801.
done. Noel et al. (1972) stated the requirement for puppies is < 88 but > 30 μg · kg body wt⁻¹ · d⁻¹. Red blood cell riboflavin concentration was used as the indicator of riboflavin status in their work. Although red blood cells are a storage site for flavins, erythrocyte riboflavin is not a good indicator of status because it does not detect riboflavin deficiency before gross clinical signs appear (Noel et al. 1972). When Noel et al. (1972) severely restricted the riboflavin intake of the bitches for 7 wk, they found a marked decrease in the red blood cell riboflavin levels along with depressed weight gain.

There is a gap in our knowledge concerning the riboflavin requirements of dogs. In addition, there have been improvements in the technology used to assess riboflavin status in animals and humans that should be applicable to dogs. Therefore, the aim of the present study was to determine the riboflavin requirement of mature dogs at maintenance using the erythrocyte glutathione reductase activity coefficient (EGRAC) as the determinant of riboflavin status.

**MATERIALS AND METHODS**

**Animal management.** Twenty adult, mixed breed dogs, 10 males and 10 females (Wildwood Kennels, Watseka, IL) were acclimated for a 2-wk period during which each dog was fed 500 g/d of a commercial diet (Hubbard, 21% Protein, Mankato, MN). The dogs were housed in individual pens in a facility that provided both a 1.37 × 1.21-m indoor pen and a connected 3.05 × 1.21-m outdoor run. The indoor area was maintained between 20 and 25°C by a gas-fired heating unit. Individual plastic sleeping pads were provided to add to thermal comfort. Continuous ventilation was maintained by a forced-air fan and duct system. Free access to water was provided.

**Experimental design.** On d 1 of the experiment, each dog was weighed and randomly assigned to one of five dietary treatments based on gender and weight in a randomized complete block design. The mean weights for Groups 1 through 5 were 16.93, 15.60, 15.83, 17.58 and 17.03, respectively (SEM = 0.496). The basal diet was formulated to meet or exceed the NRC (1985) requirements for all nutrients except riboflavin. Composition of the basal diet is given in Table 1. The lard was melted over a gas burner while the dry ingredients including the vitamin and mineral premixes were thoroughly mixed using a ribbon mixer. The lard was added to the dry ingredients and mixing was continued for 30 min. The basal diet was then shaken through a 4-mm screen and then divided into five aliquots. Each aliquot was supplemented with a different level of riboflavin to make one of the experimental diets. Each experimental diet was then mixed for an additional 30 min. The experimental diets were constructed by the addition of reagent grade riboflavin (98% pure, Aldrich Chemical, Milwaukee, WI) to the basal diet. The basal diet was analyzed for riboflavin content via microbiological assay [Hazleton Laboratories, Madison, WI].

Each dog was fed 450 g of their assigned diet per day. Coprophagy was observed in some of the dogs over the first 28 d of the experiment, so the amount of diet per day was increased to 500 g in an attempt to alleviate this problem. The feed was moistened with 155 mL of water per 500 g of feed to increase palatability.

**Data collection and analysis.** Body weights were recorded, and blood samples were taken every 28 d over the 112-d trial. The erythrocytes collected from each dog were washed following the procedure described by Nicholaids (1974) and frozen at −20°C. The principle of the erythrocyte glutathione reductase (EGR) assay is based on the use of the vitamin precursor in its biologically functional form. The enzyme EGR catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) using FAD as a coenzyme.

\[
\text{NADPH}^+ + \text{GSSG} \xrightarrow{\text{EGR-FAD}} \text{NADP}^+ + 2\text{GSH}
\]

On the day of EGRAC analysis, washed erythrocytes were thawed at 30°C. The cells were lysed with ice-
cold deionized water and centrifuged for 15 min at 1700 × g. The lysed cells were incubated at 37°C for 8 min in cuvettes with and without exogenous FAD. The change in absorbance [due to NADPH oxidation] was monitored at 340 nm as erythrocytes were incubated with exogenous FAD and was expressed as an activity coefficient (AC). The AC computation was:

\[
AC = \frac{\Delta \text{Absorbance at 340 nm with added FAD}}{\Delta \text{Absorbance without added FAD}}
\]

As such, the higher the AC, the lower the riboflavin status. The assay was validated using dog erythrocytes with an intrasay CV of 1.9% and an interassay CV of 3.9%.

The EGRAC data for each sampling day were analyzed using ANOVA appropriate for a randomized complete block design [SAS 1988]. The EGRAC of dogs fed Diet 1 was compared with those of dogs fed Diets 2 through 5 using single df comparisons. The EGRAC for dogs fed each diet were analyzed over time by using repeated measures ANOVA [SAS 1988]. Broken line analysis was applied to the EGRAC data from d 112 to determine the riboflavin requirement [Robbins et al. 1979].

**RESULTS**

Figure 1 displays the mean EGRAC for each sampling day. Before d 56 there were no significant difference among the diet groups. At d 56, the EGRAC of dogs fed Diet 1 was significantly greater than that of dogs fed Diet 5 (P < 0.05). On d 84, the mean EGRAC for dogs fed Diet 1 was significantly different (P < 0.05) from the coefficients obtained for dogs fed the other diets. The EGRAC for dogs fed Diet 1 increased significantly over time after d 56 (P < 0.05), and the EGRAC values for dogs fed Diet 3 were significantly higher (P < 0.05) at d 112 than at any other sampling day.

Results of the riboflavin analysis of the diets and the mean EGRAC for d 112 are presented in Table 2. There was a difference (P < 0.01) in mean EGRAC between dogs fed the lowest concentration of riboflavin [Diet 1] and those fed each of the higher concentrations of riboflavin [Diets 2–5]. The mean EGRAC for dogs fed Diets 2, 3, 4 and 5 were not different (P < 0.05) on d 112. The EGRAC data for d 112 and the requirement estimate is presented in Figure 2. The broken line requirement estimate for the adult dog at maintenance was 66.8 μg riboflavin·kg body wt⁻¹·d⁻¹.

**DISCUSSION**

The dogs fed Diets 1 and 3 had increasing EGRAC over time with the EGRAC indicating marginal deficiency beginning on sampling d 56 for dogs fed Diet 1. If this study had been continued, greater differences in EGRAC between dogs fed the different diets may have been evident. Dogs fed Diets 2 and 3 may have shown an increased EGRAC over a longer time period. How-
TABLE 2

Riboflavin intake and mean erythrocyte glutathione reductase activity coefficient (EGRAC) on d 112 in adult dogs fed graded levels of riboflavin

<table>
<thead>
<tr>
<th>Diet</th>
<th>Intake1,2</th>
<th>EGRAC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, Basal4</td>
<td>44.20</td>
<td>1.59a</td>
</tr>
<tr>
<td>2, as 1 + 1 mg/kg riboflavin</td>
<td>69.00</td>
<td>1.25b</td>
</tr>
<tr>
<td>3, as 1 + 2 mg/kg riboflavin</td>
<td>102.20</td>
<td>1.36b</td>
</tr>
<tr>
<td>4, as 1 + 3 mg/kg riboflavin</td>
<td>120.70</td>
<td>1.27b</td>
</tr>
<tr>
<td>5, as 1 + 4 mg/kg riboflavin</td>
<td>149.20</td>
<td>1.21b</td>
</tr>
<tr>
<td>SEM</td>
<td>8.88</td>
<td>.077</td>
</tr>
</tbody>
</table>

1 Mean feed intake [g/d] for the 112 d experiment was Diet 1 = 484; Diet 2 = 457; Diet 3 = 484; Diet 4 = 474; Diet 5 = 484; SEM = 11 g.
2 Mean riboflavin intake · kg body wt⁻¹·d⁻¹ for entire 112-d experiment.
3 Values are means (n = 4), values with different superscripts are different (P < 0.05).
4 Basal diet was analyzed and found to contain 1.7 mg/kg of riboflavin.

ever, because of the low amount of riboflavin excreted from the body (Rivlin 1984) and the large amount of riboflavin that can be stored in the body (Noel et al. 1972), the delayed increase in EGRAC is, in retrospect, not surprising. Street and Cowgill (1939) reported that > 100 days of consuming a riboflavin deficient diet were required before signs of deficiency in dogs were presented. Street et al. (1941) also stated that the development of the collapse syndrome in dogs fed a practically riboflavin-free diet did not occur until d 100–150 on test. Frank et al. (1984, 1988) did show increased EGRAC in sows soon after the beginning of their study; however, the animals used in these studies were in a different metabolic state, i.e., pregnancy and lactation, which could affect riboflavin turnover.

If the experiment had been continued, the differences in EGRAC due to diets may have been greater; however, the riboflavin requirement estimate for adult dogs suggested by this study, 66.8 µg · kg body wt⁻¹·d⁻¹, is still higher than previous studies. Street and Cowgill (1939) suggested a requirement of 25 µg of riboflavin · kg body wt⁻¹·d⁻¹ based on the collapse syndrome as the indicator of deficiency. Potter et al. (1942) extrapolated a requirement of between 30 and 50 µg · kg body wt⁻¹·d⁻¹ for adult dogs at maintenance from an estimated requirement of puppies of 60–100 µg · kg body wt⁻¹·d⁻¹ based on urinary riboflavin excretion. However, the criterion used to determine the riboflavin requirement in these studies is imperfect when compared with the sensitivity of the EGRAC method.

The EGRAC detects biochemical deficiency of riboflavin before clinical signs of deficiency occur (Bamji 1969). This method is used extensively in detecting subclinical riboflavin deficiency in humans (Bamji et al. 1991, Beutler 1969, Glattle 1970) and has been used to detect biochemical deficiency of riboflavin in swine (Esch et al. 1980, Frank et al. 1984, 1988) and rats (Dutta et al. 1988, Powers 1985). The EGRAC increased in response to low riboflavin intake in the adult dogs of this study.

The degree of stimulation in EGRAC that is required to define a deficiency in dogs is unclear. Tillotson and Baker (1972) suggested that an EGRAC between 1.3 and 1.8 is indicative of a subclinical deficiency in human subjects. An EGRAC between 1.0 and 1.3 is considered normal (Tillotson and Baker 1972). Frank et al. (1984, 1988) used similar guidelines when determining deficiency in pregnant and lactating sows and their piglets. Powers (1985) and Dutta et al. (1988) also used the same guidelines for rats. If the EGRAC obtained in this study is evaluated according to the guidelines suggested by previous authors, we find the following: dogs fed Diet 1 were severely deficient by d 112, and dogs fed Diets 2, 3, 4 and 5 had near normal EGRAC.

The aim of this study was to determine the riboflavin requirement of adult dogs at maintenance based on EGRAC assessment. Therefore, the classical signs of riboflavin deficiency were neither expected nor observed. The differences in EGRAC between dogs fed various concentrations of riboflavin were smaller than anticipated; this may be due in part to the higher than expected level of riboflavin in the basal diet. The bioavailability of the riboflavin in this experimental diet is unknown; however, Chung and Baker (1989) estimated the bioavailability of riboflavin in a corn-soybean meal diet using growing chicks to be 59.1%.
LITERATURE CITED


