

### THE VITAMIN D ASSAY OF THE "REFERENCE COD-LIVER OIL"

DURING the past four years, this laboratory has made studies of several fundamental factors<sup>1,2</sup> involved in biological assays in which chicks have been employed as experimental material. The adaptability of the chick for rickets investigations, as well as for studies of bone development, has been well established, due to the following reasons: Firstly, the disease is easily produced in chicks deprived of vitamin D; secondly, reliable experimental data of value to the poultry industry may be obtained in a short space of time; thirdly, a large number of birds may be secured from a single hatching for experimental purposes; and fourthly, the results obtained with chicks are particularly suitable for the vitamin D evaluation of antirachitic supplements, especially cod-liver oils and other fish oils which are marketed for poultry.

Largely owing to the great sensitivity of the chick to rickets, there has been an ever-growing interest by nutrition investigators in this species as a satisfactory medium for vitamin D assay studies. The authors are of the opinion, due to the importance of the chick as a test animal, that some practical measure for determining the difference in bone calcification and skeletal development between the rat and the chick should be made. With this object in mind, an experimental study was planned.

A quantity (120 ccs) of the "Reference cod-liver oil" was purchased from the United States Pharmacopoeia Vitamin Advisory Board. This Reference cod-liver oil of known vitamin D potency was fed to six groups of day-old White Leghorn chicks for a period of four weeks, using the technique developed for the vitamin D assay, which was adopted by the Association of Official Agricultural Chemists.<sup>3</sup> A seventh group of fifteen birds served as the "negative control" lot. At the same time, three samples of commercial poultry cod-liver oils were assayed according to the routine technique employed in this laboratory.<sup>4</sup>

The results of this preliminary feeding trial showed that each chick required between 80 and 135 International vitamin D rat units to produce satisfactory calcification as observed by the criteria of normal growth and an average bone ash content in excess

of 45 per cent. This requirement was calculated on the basis of the total length (four weeks) of the feeding period. The experiments lend support to the theory that the vitamin D requirement of the chick is enormous when compared with the requirement of the rat for the antirachitic substance in cod-liver oil. It was also noted from these investigations that the Reference cod-liver oil, containing 95 International vitamin D rat units per gram, was considerably poorer as a source of antirachitic activity than a number of commercial poultry cod-liver oils, which had been assayed for vitamin D content.

The experimental studies mentioned were completed in March of this year. It is the purpose of the authors to repeat the bioassay of the Reference oil at different seasons of the year to determine, if possible, the antirachitic evaluation of cod-liver oils and other fish oils in terms of International vitamin D units. From the standpoint of control testing of vitamin D supplements, the requirement of the chick for vitamin D, with respect to the seasonal variation in average bone ash content throughout the year, should be evaluated so that results obtained in bioassays may be better interpreted. When the complete data have been obtained, the results will be released for publication.

The aim of the present communication is to merely state the problem which is involved in reducing the chick assay of antirachitic supplements to terms of International rat units. On the basis of the foregoing discussion, the opportunity has presented itself to invite the cooperation of other workers in chick biological laboratories who may be interested in this type of study.

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- <sup>1</sup> L. L. Lachat, H. A. Halvorson and L. S. Palmer, "Studies Relative to the Estimation of Vitamin D, I," *Jour. Assoc. Official Agr. Chem.*, 15: 660-675, 1932.
- <sup>2</sup> L. L. Lachat, "Studies Relative to the Estimation of Vitamin D, II. Effect of Seasonal Variation and Sex upon Calcification in Rachitic Chicks," *Poultry Sci.* (in press).
- <sup>3</sup> W. B. Griem, "Vitamin D Assay by Preventive Biological Test," *Jour. Assoc. Official Agr. Chem.*, 17: 69, 1934.
- <sup>4</sup> H. A. Halvorson and L. L. Lachat, "Antirachitic Activity of Vitamin D Supplements for Poultry," State of Minnesota Dept. of Agr. Dairy and Food, April, 1934, 16 pps.