

NUTRITIONAL ASSAY

Ash Content of Chick Beak for Vitamin D Assays

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The AOAC method for determining vitamin D in supplements intended for poultry feeding compares the percentage ash of dried and extracted tibias from groups of chicks grown under specified conditions. A more rapid and satisfactory procedure has been developed using beaks, which are more easily and quickly prepared for analysis than tibias. Comparisons also were made with toe-ash methods. Differences in percentage ash of both upper and lower beaks from chicks receiving the basal ration and basal ration plus various added amounts of vitamin D were as large as or larger than differences in percentage ash of tibias and of toes from the same chicks. Duplicate determinations on beaks were as satisfactory as those on tibias.

VITAMIN D IN POULTRY FEED SUPPLEMENTS is determined in the AOAC method (7) by making comparisons of the percentage ash of dried and extracted tibias. Some workers, however, have proposed using the percentage ash of toes, either fresh (4, 6) or dried and extracted (3), as an alternative procedure. Because softness of beaks appeared to vary with vitamin D contents of feed consumed by chicks, studies were undertaken to determine whether the ash content of beaks could be used in the assay instead of that of tibias or toes, and if there were advantages in the proposed procedure. This paper reports results of an investigation of this problem.

Methods

Procedures of the AOAC method (7) were followed in preparation of rations and in selection and care of chicks. Chicks were housed in wire-floored brooders under controlled temperature conditions and protected from sunlight. In each of seven trials, one group of chicks received basal ration only and two or more additional groups received basal ration plus graded levels of vitamin D, as shown in Tables I and II. The source of vitamin D was U. S. P. XIV reference oil. In some instances the number of chicks placed in each group was smaller than the number required by the official assay. In three trials the assay period was 12 days in-

stead of 21, in order to determine whether an assay based on ash contents of beaks of chicks fed for the shorter period would be satisfactory.

Tibias were prepared for ashing in the usual manner (7), fresh toes, as proposed by Campbell, Migicovsky, and Emslie (4), and dried and extracted toes, as suggested by Baird and MacMillan (3). The lower beak was removed by opening the mouth of the dead chick and sliding the upper blade of scissors under and backward to the attachment of the tongue and cutting. The upper beak was removed by cutting diagonally from below and in back of the nostril upward and in front of it, following the easily discernible anatomic line separating the harder fore part from the rest of the beak. The fleshy parts, skin, etc., remaining on the beaks were trimmed away and the beaks were wiped with a dry cloth. Beaks were dried, extracted, and ashed in the same manner as were tibias.

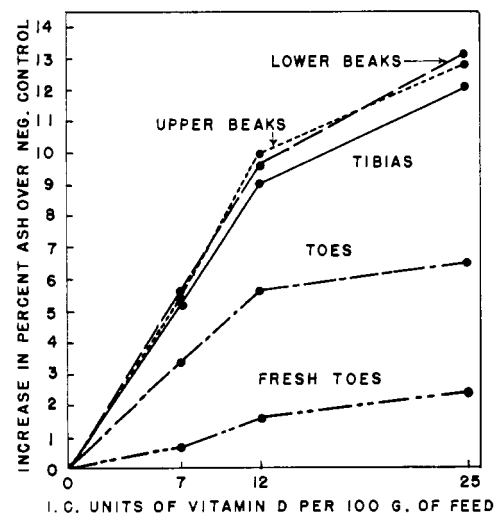
Results and Discussion

Percentage ash of upper and lower beaks of chicks receiving various amounts of vitamin D during 21- and 12-day assay periods is shown in Tables I and II. Percentage ash of tibias, toes, and fresh toes from the same chicks is shown for comparative purposes. In each of the first three trials, differences in percentage ash of beaks from chicks that received 0 and 25 units of vitamin D per 100 grams of feed were greater than those of tibias from the same chicks (Table I). In trial IV (0 to 12 units of vitamin D) the same

was true with respect to upper beaks, but the difference in percentage ash of lower beaks was 0.6 less than that of tibias. Data from the first four trials (Table I) have been combined and plotted (Figure 1) to show increases in percentage ash as amounts of vitamin D in feed were increased. Results are similar for both upper and lower beaks at each level of supplementation. Increases in percentage ash of tibias were slightly less, and of toes markedly less, than those of beaks. When data were examined for sensitivity

Figure 1. Increases of per cent ash in chick bones

Increases in bones of chicks that received added amounts of vitamin D in feed above ash in bones of chicks fed basal feed only (negative control)



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Table I. Relation of Ash of Chick Bones to Vitamin D Content of Feed

| Trial and Group | I.C. Units Vitamin D per 100 G. Feed | Feeding Period, Days | No. of Chicks ^a | Percentage Ash | | | | |
|-----------------------|--------------------------------------|----------------------|----------------------------|----------------|------------|-------|------|-----------|
| | | | | Upper beak | Lower beak | Tibia | Toe | Fresh toe |
| Trial I | | | | | | | | |
| A | 0 | 21 | 12 | 13.5 | 22.1 | 32.1 | 10.6 | 3.6 |
| B | 7 | 21 | 20 | 19.4 | 27.9 | 39.6 | 14.8 | 3.8 |
| C | 12 | 21 | 19 | 24.2 | 33.3 | 42.1 | 17.2 | 4.5 |
| D | 25 | 21 | 10 | 27.5 | 36.0 | 45.4 | 18.0 | 5.7 |
| Trial II | | | | | | | | |
| A | 0 | 21 | 13 | 16.4 | 23.9 | 31.3 | 11.7 | 3.5 |
| B | 7 | 21 | 20 | 21.0 | 29.1 | 35.2 | 14.0 | 4.3 |
| C | 12 | 21 | 19 | 25.5 | 32.8 | 39.0 | 16.4 | 5.3 |
| D | 25 | 21 | 13 | 27.4 | 34.9 | 41.9 | 17.0 | 6.0 |
| Trial III | | | | | | | | |
| A | 0 | 21 | 13 | 14.2 | 21.5 | 30.3 | 10.9 | 4.2 |
| B | 7 | 21 | 27 | 20.5 | 29.3 | 37.2 | 14.8 | 5.0 |
| C | 12 | 21 | 27 | 25.5 | 34.1 | 41.0 | 17.2 | 6.0 |
| D | 25 | 21 | 14 | 27.7 | 35.9 | 42.3 | 17.7 | 6.4 |
| Trial IV ^b | | | | | | | | |
| A | 0 | 21 | 11 | 15.5 | 23.1 | 33.6 | 10.9 | 3.5 |
| B | 7 | 21 | 13 | 20.8 | 28.3 | 37.9 | 14.1 | 4.6 |
| B' | 7 | 21 | 14 | 20.2 | 26.8 | 36.6 | 13.9 | 4.4 |
| C | 12 | 21 | 18 | 24.5 | 31.2 | 42.2 | 16.0 | 5.3 |
| C' | 12 | 21 | 17 | 24.9 | 30.3 | 41.4 | 16.0 | 5.1 |

^a Number surviving. Death losses insignificant except in trial IV, in which losses the first 4 days were high, apparently the result of infection of newly hatched chicks.

^b B and B' and C and C' are duplicate groups placed on experiment at the same time.

percentage ash of bones from chicks receiving no vitamin D and various added amounts (Table I and Figure 1), use of either the upper or the lower beak is as satisfactory as is use of the tibia for studying the degree of calcification and vitamin D content of supplements. Beaks, particularly the lower, are more easily prepared for ashing. Beaks can be quickly removed and trimmed of adhering soft tissue. They require no boiling and tedious removal of flesh; there is no necessity of crushing them prior to extraction. As beaks are less bulky to handle than tibias, smaller extraction and ashing apparatus may be used. Toes may be prepared for ashing more easily than either tibias or beaks, but percentage ash and differences in percentage ash of toes from chicks receiving various levels of vitamin D are small. The authors prefer use of lower beaks in the assay.

Literature Cited

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(6), both lower and upper beaks were more sensitive for the assay than tibias and upper beaks were superior to toes.

To check agreement of findings in duplicated determinations, two groups of chicks were given vitamin D at each of the levels, 7 and 12 units per 100 grams of feed (trial IV). At both levels of supplementation the differences in the percentage ash of upper beaks from duplicate groups of chicks were less than, and of lower beaks similar to, those of tibias. In trials II and III all of the birds fed a single assay level of vitamin D were grown together, but the bones were divided at random into two lots before ashing. If differences in percentage ash of these divided lots (data not shown) and those of duplicate determinations of trial IV referred to above are averaged, values of 1.05, 0.53, 0.28, 1.26, and 1.04% are obtained for tibias, toes, fresh toes, upper beaks, and lower beaks, respectively. Similar calculations on duplicate determinations of tibia ash in two collaborative studies (2, 7) yielded values of 1.134 and 0.975%. Average differences calculated from duplicate tibia and toe data in two earlier reports were 1.35 and 0.36% (3) and 1.33 and 0.63%, respectively (6).

Percentage ash of bones from chicks fed for only a 12-day period are shown in Table II. The shortened assay did not yield so satisfactory results as the 21-day assay, for differences in percentage ash of beaks and of other bones from chicks receiving no vitamin D and those receiving the largest amounts in each of the trials are relatively small. This is in accord with findings of DeWitt *et al.* (5)

in shortened assay periods using tibia ash for measuring response. In 12-day assays ash contents of bones did not so consistently increase with increasing amounts of vitamin D in the feed as in 21-day assays. For rapid survey purposes the shortened assay period using either beaks or tibias might sometimes be used to advantage.

The weights of dried and extracted lower beaks averaged about 16% and of upper beaks, 13% of that of tibias. The ashes of these beaks weighed about 13 and 8%, respectively, of that of tibias. Similar comparisons of dry, extracted toes and toe ash are 23 and 9%, respectively.

As judged by data on differences in

Table II. Relation of Ash of Chick Bones to Vitamin D Content of Feed

| Trial and Group | I.C. Units Vitamin D per 100 G. Feed | Feeding Period, Days | No. of Chicks | Percentage Ash | | | | |
|-----------------|--------------------------------------|----------------------|---------------|----------------|------------|-------|------|-----------|
| | | | | Upper beak | Lower beak | Tibia | Toe | Fresh toe |
| Trial 5 | | | | | | | | |
| A | 0 | 12 | 8 | 12.0 | 17.1 | 27.7 | 9.6 | 2.7 |
| B | 3 | 12 | 7 | 11.6 | 17.2 | 27.6 | 9.5 | 2.7 |
| C | 6 | 12 | 7 | 13.0 | 17.6 | 27.6 | 9.7 | 2.7 |
| D | 10 | 12 | 7 | 14.1 | 19.1 | 28.9 | 10.4 | 2.9 |
| E | 20 | 12 | 7 | 16.7 | 21.3 | 32.3 | 12.1 | 3.2 |
| Trial 6 | | | | | | | | |
| A | 0 | 12 | 13 | 17.0 | 22.1 | 35.4 | 13.0 | 4.1 |
| B | 5 | 12 | 13 | 17.6 | 23.1 | 35.5 | 12.7 | 4.1 |
| C | 10 | 12 | 13 | 19.9 | 24.0 | 37.8 | 14.3 | 4.6 |
| D | 17 | 12 | 13 | 21.4 | 25.8 | 39.7 | 15.6 | 4.7 |
| Trial 7 | | | | | | | | |
| A | 0 | 12 | 7 | 16.7 | 22.0 | 35.4 | 14.2 | 4.0 |
| B | 5 | 12 | 7 | 18.7 | 24.1 | 41.0 | 14.9 | 4.1 |
| C | 10 | 12 | 6 | 21.5 | 26.4 | 41.6 | 17.3 | 4.6 |
| D | 17 | 12 | 6 | 21.1 | 25.7 | 42.1 | 17.5 | 5.0 |