no particular toxicity to the eggs or to the free-swimming nauplii.

1. Lovelock, J. W., Bishop, M. W. H., Nature, 1959, v183, 1394.
2. Ashwood-Smith, M. J., ibid., 1961, v190, 1204.
3. Jones, R. C., Austral. J. Biol. Sci., 1965, v18, 887.
4. Brown, B. L., Nagle, S. C., Jr., Science, 1965, v149, 1266.
5. Bouroncle, B. A., Proc. Soc. Exp. Biol. & Med., 1965, v119, 958.
6. Hellman, A., Farrelly, J. G., Martin, D. H., Nature, 1967, v213, 982.
7. Franz, T. J., van Bruggen, J. T., Ann. N. Y. Acad. Sci., 1967, v141, 302.
8. Jacob, S. W., Bischel, M., Herschler, R. J., Curr. Therapeut. Res., 1964, v6, 193.
9. Kligman, A., J. Am. Med. Assn., 1965, v193, 140.
10. McDermont, H. L., Finkbeiner, A. J., Wills, M. J., Heggie, R. M., Canad. J. Physiol. Pharmacol., 1967, v45, 299.
11. Clegg, J. S., J. Exp. Biol., 1964, v41, 879.
12. Martin, E., Wilber, B., Am. J. Physiol., 1921, v55, 290.
13. Boone, E., Baas-Becking, L. G. M., J. Gen. Physiol., 1931, v14, 753.
14. Croghan, P. C., J. Exp. Biol., 1958, v35, 213.

Received May 23, 1967. P.S.E.B.M., 1967, v126.

Breaking Strength of Chick Bones as an Indication of Dietary Calcium and Phosphorus Adequacy.*† (32458)

L. O. ROWLAND, JR., R. H. HARMS, H. R. WILSON, I. J. ROSS‡ AND JACK L. FRY
Florida Experiment Station, Gainesville, Florida

It is an accepted fact that the levels of dietary calcium and phosphorus regulate bone ash content. Harms *et al*(1) reported that maximum growth could be attained with much lower levels of calcium or phosphorus than those indicated to be required(2), provided the calcium-phosphorus ratio was optimum. However, in many instances bone ash was increased by higher dietary levels of the two minerals than were required for normal growth. The value of the increased bone ash has been questioned.

In most calcium or phosphorus assays, tibia ash has been used as the main criterion for evaluation of dietary adequacy(3). The purpose of this study was to determine whether the increased bone ash was beneficial to the chick for providing greater bone strength, and to develop a method for measuring bone strength.

Procedure. The basal diet used in this study contained .26% calcium and .30% phosphorus (Table I). Nine experimental diets were fed which involved 3 levels of calcium at each of 3 levels of phosphorus. Calcium and phosphorus levels were chosen which had been found to produce a wide variation in tibia bone ash(1). Reagent grade monosodium phosphate ($NaH_2PO_4 \cdot H_2O$) and reagent grade calcium carbonate ($CaCO_3$) were used to supplement the basal diet.

TABLE I. Composition of Basal Diet.

Ingredient	%
Degerminated corn meal	51.70
Cerelose	5.40
Soybean meal (50% protein)	34.00
Alfalfa meal (20% protein)	3.00
Iodized salt	.40
Micro-ingredients*	.50
Variable†	5.00
% Phosphorus	.30
% Calcium	.26

* Supplied per kg of diet: 7,000 I.U. vit A, 2200 I.C.U. vit D_3, 770 mg choline chloride, 40 mg niacin, 4.4 mg riboflavin, 20 mg pantothenic acid, 22 μg vit B_{12}, 125 mg ethoxyquin, 20 mg iron, 2 mg copper, 200 μg cobalt, 1.1 mg iodine, 100 μg zinc, 175 mg manganese and 125 μg zoalene.

† Composed of monosodium phosphate, calcium carbonate and builder's sand.

* Florida Agr. Exp. Sta. Journal Series No. 2699.

† This work was supported in part by a grant-in-aid from Borden Chemical Co. (Smith-Douglas Division), Norfolk, Va.

‡ Present Address: Dept. of Agr. Engineering, Univ. of Kentucky, Lexington, Ky.

At one day of age, 360 egg production type cockerels were randomized into 18 pens of 20 chicks each. Two pens were fed each of the diets for a period of 28 days. After the treatment period, the 5 largest and 5 smallest cockerels were discarded from each of the 18 pens in an effort to achieve better uniformity. Both legs were removed from the remaining 20 chicks fed each diet and the tibiae were defleshed after cooking for approximately 5 minutes in boiling water.

The force required to break each tibia was measured by use of an Allo-Kramer shear press§ (Model #SP12 with Recorder #E2EZ). An attachment for the shear press was designed whereby pressure could be placed on the midpoint of the tibia which was supported near each end. The bottom area of the attachment was 3 mm × 19 mm and had been machined from a 19 mm diameter steel rod (Fig. 1 and 2). An adult hen tibia could be supported on a steel box (Fig. 1) previously made for another use; the square box was 87 mm wide inside, and had an inside depth of 70 mm and a wall thickness of 5 mm. For the chick bones used in this study an adjustable support (Fig. 3) was designed to fit

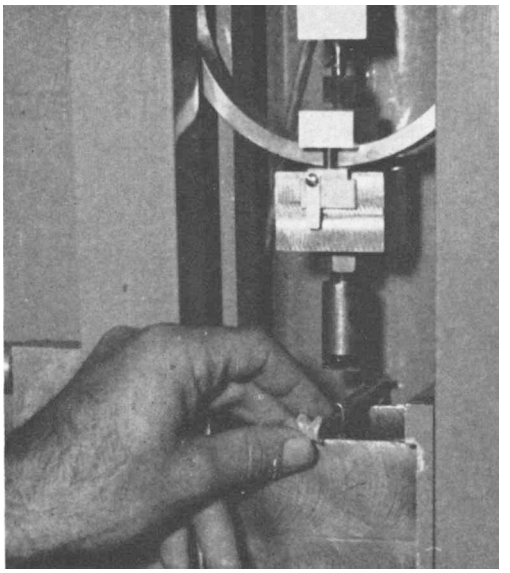

FIG. 1. Illustration of bone strength determination using attachments for the Allo-Kramer shear press.

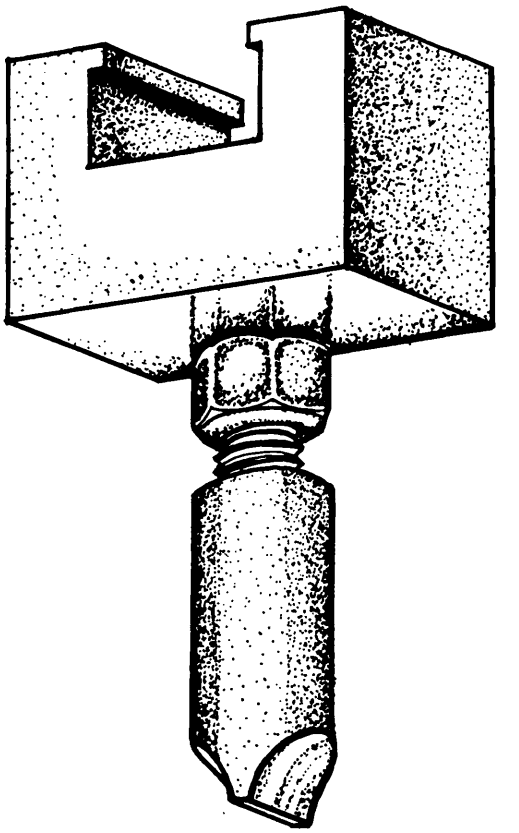

FIG. 2. Bone breakage attachment for the Allo-Kramer shear press.

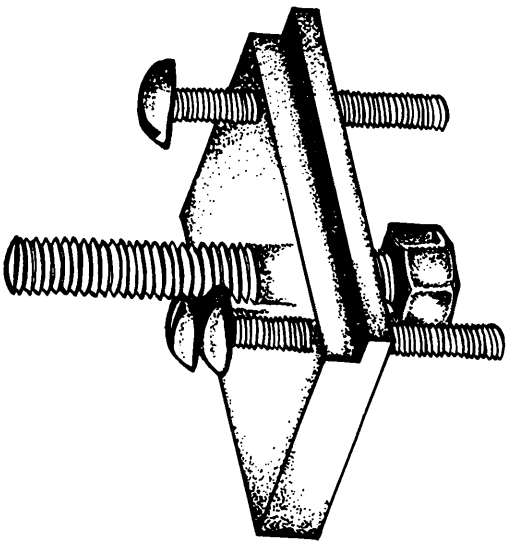

FIG. 3. Bone support attachment for the Allo-Kramer shear press.

§ Allo Precision Metals Engineering, Inc., Rockville, Md.

TABLE II. Body Wt, Bone Ash and Breaking Strength of Bones from Chicks Fed 3 Levels of Phosphorus at 3 Levels of Calcium.

Phosphorus*(%)	Calcium*(%)	Body wt (g)	Bone ash† (%)	Breaking strength† (lb)
.32	.42	238	42.45^{e}	8.85^{e}
.32	.38	257	42.51^{e}	9.92^{d}
.33	.51	280	46.14^{c}	11.37^{c}
.40	.43	234	42.01^{e}	8.73^{e}
.41	.52	282	45.02^{d}	11.30^{c}
.42	.66	296	48.35^{b}	12.74^{b}
.60	.42	224	42.08^{e}	8.62^{e}
.59	.67	275	47.28^{bc}	12.22^{b}
.60	.83	304	50.88^{a}	14.71^{a}

* Calcium and phosphorus analyses were made by Borden Chemical Co., Smith-Douglass Division, Plant City, Fla.

† Means with different superscripts are significantly different ($P<.05$) according to Duncan's Multiple Range Test.

inside the box and permitted an unlimited selection of support widths. In this study each tibia was supported near the ends approximately 14 mm from the midpoint at which the force was applied. The press descended at the rate of one centimeter each 5 seconds. The maximum force required to break each bone was read from the chart of the shear press recorder. The bone fragments were collected, defatted, dried and ashed(4).

Results and discussion. Tibia ash was significantly increased by increasing the dietary phosphorus or by increasing the calcium level at each level of phosphorus (Table II). Based on previous reports with chicks(1) and rats (5), these findings were expected. Breaking strength of the tibiae was increased each time bone ash was increased. The correlation coefficient for tibia ash and tibia breaking strength was +.63 when calculations were made on an individual bird basis. However, a correlation coefficient of +.98 was found when the average tibia ash was correlated with the average breaking strength.

The high correlation between tibia ash and tibia breaking strength, as measured by the modified Allo-Kramer shear press, would indicate that breaking strength of bones is as useful a measurement of the calcium and phosphorus content of a diet as is tibia ash. The use of breaking strength as a criterion of mineral level in the diet could be a very useful tool in calcium and phosphorus studies. In this determination it would not be necessary to free the bone of all adhering flesh and hence there would be a considerable saving in the time required to prepare the bones. Also the time required to determine the breaking strength of bones is considerably less than that required for ashing. Because of these savings it would be possible to make more measurements per treatment, thus improving the accuracy of the assay.

This method of breaking bones appears to give a good indication of bone strength. Therefore, it is suggested that this procedure will be of considerable benefit in studying factors affecting bone fragility.

Summary. An attachment was designed which made it possible to use the Allo-Kramer shear press to determine the breaking strength of the tibia of 4-week old chicks. Breaking strength determined in this manner was found to be highly correlated with tibia ash. It is suggested that the breaking strength is as good a measure of dietary calcium and phosphorus levels as is tibia ash. Also the breaking strength of bones as determined in this manner may be useful in evaluating factors influencing fragility of bones.

1. Harms, R. H., Waldroup, P. W., Damron, B. L., Poultry Sci., 1967, v46, in press.

2. National Research Council, Nat. Acad. Sci. Pub. 1345.

3. Waldroup, P. W., Ammerman, C. B., Harms, R. H., Poultry Sci., 1965, v44, 1302.

4. AOAC, Methods of Analysis, 1965.

5. Shah, B. G., Krishnarao, G. V. G., Draper, H. H., J. Nutr., 1967, v92, 30.

6. Duncan, M., Biometrics, 1955, v11, 1.

Received June 5, 1967. P.S.E.B.M., 1967, v126.