ually and 12 were killed and their tibia were separated, cleaned, dried and weighed. Bone ash of tibia was also determined. The results are shown in Table 3. As the quail increased in body weight with age, the relative weight of tibia decreased. However, the mineralization of tibia increased with age. In mature quail of almost 4 months of age, the bone ash values of 64.02 ± 0.68% were observed.

It appears that 50 p.p.m. fluoride ion in water did not influence the body weights or the dried tibia weight per 100 gm. body weight at any age over the 6 weeks of the experiment.

The bone ash is slightly improved with fluoride in water but the data are not significantly different. A difference in bone ash was observed for chickens by feeding diets containing fluoride in diet (Smith *et al.*, 1970) but quail data differ in this respect.

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CHEMICAL *VERSUS* CHICK BIOASSAY FOR PHOSPHORUS AVAILABILITY OF FEED GRADE SOURCES[1]

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ABSTRACT Seven feed grade phosphates were assayed biologically using tibia bone ash as the response criterion. Phosphorus solubility in (1) 0.4% hydrochloric acid, (2) 2% citric acid and (3) neutral ammonium citrate was determined for each sample. There was very little, if any, agreement between phosphorus solubility in the dilute acid solutions and bio-availability. Of the three experimental tests, solubility in 0.4% hydrochloric acid appeared to be the most promising for predicting bio-availability, but even with this method the agreement with bioassay results was poor.

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Reynolds *et al.* (1944) used dilute hydrochloric acid solutions as a solvent for phosphate in an attempt to develop chemical methods which would accurately predict phosphorus availability for the chick. Hill *et al.* (1945) determined the solubilities of various phosphates in several solvents and concluded that dicalcium phosphate has a relatively high phosphorus solubility. Bird *et al.* (1945) showed that there was a high correlation between solu-

[1] Mississippi Agricultural and Forestry Experiment Station Journal Article No. 2454.

TABLE 1.—*Chemical solubility of the phosphorus in feed grade phosphate versus relative biological availability*

Test	Defl. phosphate					Mono/dical. phos.	Dical. phos.
	1	2	3	4	5		
Total phosphorus	18.1	18.0	18.3	18.3	18.2	21.4	19.0
Solubility, %							
0.4% HCl	93.9	97.8	96.7	99.5	97.3	94.4	99.5
2.0% citrate	79.6	81.1	66.7	74.3	81.4	93.9	99.5
Neutral amm. citrate	81.2	77.2	60.1	42.6	77.6	95.3	98.9
Bio-availability[1]	80.0	85.0	88.0	92.0	83.0	101.0	85.0

[1] Relative biological availability compared to AR monosodium phosphate as the standard and using bone ash as the response criterion.

bility and availability of the phosphorus in various supplements. In spite of these favorable reports, no chemical test has been accepted to estimate phosphorus availability. The bioassay is still routinely used by researchers to measure relative phosphorus availability.

The present study was conducted to reinvestigate the possibility of using phosphorus solubility in dilute acids as an indicator of bio-availability. To facilitate this comparison and to simulate the possible influence variation during processing may have on phosphorus availability, five samples of defluorinated phosphate were obtained which had been prepared using different heat processing treatments. A sample of predominantly dicalcium phosphate and a sample containing a mixture of mono and dicalcium phosphate were also subjected to all tests. All samples were analyzed for total phosphorus and phosphorus solubility in 0.4% HCl, 2% citric acid and neutral ammonium citrate. The samples were also subjected to a chick phosphorus bioassay as previously described (Dilworth and Day, 1964).

Results obtained from these tests are presented in Table 1. Phosphorus solubility was greatest in 0.4% HCl, ranging from a low of 94.4% for the mono/dicalcium phosphate sample to 99.5% for the dicalcium phosphate sample. The greatest range in phosphorus solubility was with neutral ammonium citrate, ranging from 42.6% for defluorinated phosphate sample 4 to 98.9% for dicalcium phosphate. With these great differences in dilute acid solubility, differences in bio-availability might be expected. However, comparing results on a group basis clearly shows that acid solubility does not correspond to biological availability. Dicalcium phosphate exhibited higher phosphorus solubility in all chemical tests than did the mono/dicalcium phosphate mixture. Yet, the chick bioassay data indicated that the phosphorus in the mono/dicalcium phosphate mixture was more available than that in the predominantly dicalcium phosphate sample—101 *versus* 85%. Similarly, defluorinated phosphate samples had the lowest solubility in citrate, samples 3 and 4, showed the highest relative bioassay values. There appeared to be an inverse relationship between solubility in neutral ammonium citrate and biological availability. These data indicated that phosphorus solubility in dilute acids cannot be used to predict bio-availability.

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EFFECT OF SIMULTANEOUS FEEDING OF AFLATOXIN AND RUBRATOXIN TO CHICKENS

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ABSTRACT A 2 × 2 factorial experiment in broiler chicks utilizing dietary aflatoxin (2.5 p.p.m.) and rubratoxin (500 p.p.m.) failed to reveal any interaction on growth rate, relative organ weights, hemoglobin, serum cholesterol, and serum total lipid despite a previous report of an interaction of these toxins in rats.

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There are some mycotoxicoses in which several different fungi and thus presumably several different toxins have been implicated. Examples are the hemorrhagic anemia syndrome of chickens (Forgacs and Carll, 1962) and alimentary toxic aleukia of humans and animals (Mayer, 1953). Nevertheless, mycotoxins usually are studied individually. Perhaps as a result, hemorrhagic anemia syndrome has not been reproduced in the laboratory (Wyatt and Hamilton, 1972). The only investigation in the literature that used a combination of mycotoxins was a brief report by Wogan *et al.* (1971) that simultaneous injections of aflatoxin and rubratoxin in rats resulted in interactions; although Hamilton and Harris (1971) did observe an interaction in chickens between dietary aflatoxin and the toxic effects of saline drinking water. Because the organisms that produce aflatoxin and rubratoxin have been implicated in the hemorrhagic anemia syndrome (Forgacs and Carll, 1962), we tested aflatoxin and rubratoxin for possible interactions in chickens.

Aflatoxin was produced by the methods given by Hamilton and Harris (1971) and rubratoxin was produced by the methods given by Wyatt and Hamilton (1972). Day-old male broiler chicks from a commercial source were brooded in an electrically heated battery with water and a commercial starter mash available *ad libitum*. There were 4 groups of 5 birds per treatment. The experimental design was a 2 × 2 factorial for the presence and absence of dietary aflatoxin (2.5 p.p.m.) and rubratoxin (500 p.p.m.). These levels are the minimal levels that reduce growth rate (Smith and Hamilton, 1970; Wyatt and Hamilton, 1972) and were selected to permit easy detection of interactions. The experiment was terminated after 3 weeks at which time blood serum and body and organ weights were obtained. Serum proteins