BIOAVAILABILITY OF PHOSPHORUS FROM VARIOUS SOURCES FOR YOUNG TURKEYS

by

Manop Potchanakorn

Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Animal Science
(Poultry Nutrition)

APPROVED:

L. M. Potter, Chairman

G. E. Bunce

C. E. Polan

K. E. Webb, Jr.

B. A. Watkins

December, 1988

Blacksburg, Virginia

BIOAVAILABILITY OF PHOSPHORUS FROM VARIOUS SOURCES

FOR YOUNG TURKEYS

by

Manop Potchanakorn

Committee Chairman: L. M. Potter
Animal Science
(Poultry Nutrition)

(ABSTRACT)

Three series of experiments were conducted to determine the relative bioavailability of various phosphate products with an emphasis on the defluorinated phosphates. In Series I, phosphorus in commercial mono/dicalcium phosphates, commercial dicalcium phosphates, commercial defluorinated phosphates, and experimental defluorinated phosphates were found to be 93, 81, 70, and 76% as available as that in dicalcium phosphate dihydrate, respectively, using 3-week body weight and toe ash in a nonlinear regression analysis.

In Series II, phosphorus in a commercial defluorinated phosphate was compared directly with that in a commercial dicalcium phosphate using poult from 1 to 4 weeks of age. No significant differences were found between the two products which may be attributed partly to the feeding of the starter diet during the pre-experimental period. The commercial defluorinated phosphate was numerically less available than the commercial dicalcium phosphate in all cases.

In Series III, phosphorus in various defluorinated phosphates was compared with that in a dicalcium phosphate dihydrate. Ash of dry

unextracted tibia, ash of dry toe, and 0 to 3-week body weight gain as responses to added phosphorus followed a sigmoidal regression model while 0 to 2-week body weight gain followed an asymptotic model in Experiment 1. The defluorinated phosphate was found to be 53% as available as the standard.

In Experiment 2, an experimental defluorinated phosphate from Series I which was found to be 76% as available as the standard was found to be 62% as available as the standard used in Series III, indicating differences in bioavailability of the two standards. In Experiment 3, two commercial defluorinated phosphates were found to have equal relative biological values, 67.2 and 67.6% as compared with the standard using poults from 0 to 2 weeks of age, and three experimental defluorinated phosphates 51.7, 74.0, and 88.2% with the product with the fine particles having the highest relative biological value.

In cases where the sigmoidal regression gave a significantly better fit to the data than the asymptotic model, the relative biological values and the associated confidence limits from the asymptotic model were equal to those from the sigmoidal model.

ACKNOWLEDGMENTS

A sincere appreciation is expressed to Dr. Lawrence M. Potter for his guidance, support, patience, and encouragement in all stages of this study. Thanks are extended to Drs. G. E. Bunce, C. E. Polan, K. E. Webb, Jr., and B. A. Watkins for their assistance and critical review of this dissertation. A warm appreciation is extended to Dr. E. T. Kornegay for his helpful suggestions during the preparation of this dissertation.

Special thanks are extended to Barbara Self, Kim Williams, Jim Shelton, Mike Blair, and John Blake for their assistance in the collection and analysis of the data. I would also like to thank the Turkey Research Center crew of Lee Roy Barnett, Eugene Long, Dale Shumate, and Benny Surface for their help. A special thank you to Dr. R. M. Hulet, Dr. A. T. Leighton, Jr., S. H. Becker, and R. W. Manley for their friendship and encouragement over the years.

Great appreciation is expressed to Steve Auman and the Texasgulf Chemical Co. for the financial support and especially for the freedom allowed in the analyses and interpretations of the data.

A very special appreciation is extended to Drs. B. R. Moss, C. W. Newman, and R. K. Newman for their guidance and encouragement during my years at Montana State University.

This dissertation is dedicated to my parents whose love and support were most essential throughout my educational process.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure | Page

<u>Figure</u> <u>Page</u>

INTRODUCTION

Phosphorus has been extensively evaluated as an essential nutrient in diets of poultry and represents the third most expensive nutrient following energy and protein. Even in the presence of some contrary evidence, the biological values of phosphorus from different inorganic phosphate sources have been considered by many nutritionists to be equal or nearly equal and the price of a phosphate product has been based on its phosphorus content.

Differences in relative biological values among commercial phosphorus sources have been reported (Nelson and Walker, 1964; Edwards, 1968; Pensack, 1974; McGillivray, 1978). In general, the monocalcium phosphates tend to have a higher relative biological value than do the dicalcium phosphates and the latter higher than the defluorinated phosphates.

Recently, Waibel *et al*. (1984) reported significant differences in bioavailability of phosphorus from various sources for turkey poults. Using slope ratio bioassay and a purified grade calcium phosphate in the monobasic and monohydrate form as a reference standard, they found phosphorus from mono/dicalcium phosphates, dicalcium phosphates, and defluorinated phosphates to be 93.6 ± 7.6, 88.3 ± 8.2, and 70.2 ± 7.2% as available as that from the standard, respectively.

Slope ratio bioassay (Finney, 1978) has been the analysis of choice for determination of relative bioavailability of feed ingredients. Park (1970) defined growth response as being an asymptotic function of feed intake and a sigmoidal function of age. Noll *et al*. (1984) fitted an asymptotic function to a dose response of added methionine from various methionine compounds to determine the relative bioavailability of those compounds. Robbins et al. (1979) stated that some growth responses are evidently sigmoidal and that the asymptotic responses may be the upper portion of the sigmoidal curve with the lower doses not tested or not attainable.

A series of experiments was conducted to:

1. determine the relative bioavailability of various phosphate sources commonly used in turkey diets,

2. determine the relative bioavailability of some experimental defluorinated phosphate sources proposed for commercial production, and

3. define growth response as a function of added phosphorus to obtain a more precise measurement of relative bioavailability.

REVIEW OF LITERATURE

Reviews of the literature on requirement and utilization of phosphorus by poultry have been prepared by Fritz (1969), Peeler (1972), Harms and Damron (1977), McGillivray (1978), De Groote (1983), Roland (1985), Sullivan (1985), and Waldroup (1985).

Since phosphorus plays an important role in bone calcification, the A.O.A.C. methods for vitamin D bioassay have been modified for use in phosphorus bioassay. Two official A.O.A.C. methods for vitamin D_3 bioassay involve the use of ash of dried extracted tibia and of ash of dried unextracted toe (A.O.A.C., 1984).

Tibia ash

In most studies, body weight and tibia ash were the measurements of choice to evaluate availability of phosphorus sources. Nelson and Walker (1964) reported that tibia ash percentage was a more sensitive criterion than body weight, the latter requiring about six times as many observations to secure the same precision. Nelson (1967) summarized the studies on availability of the phytate phosphorus and found the percent bone ash to be one of the most sensitive practical criteria for measuring phosphorus availability. Body weight gain, however, was found to be a less accurate measure of phosphorus utilization with some researchers reporting that the phytate phosphorus was more available for growth than for bone ash. The favorable growth response without the accompanying bone ash response was normally observed to be related to dietary calcium levels.

Gillis *et al*. (1954), in the first quantitative study on biological value of inorganic phosphates, used tibia ash as the sole criterion for determining phosphorus availability in White Leghorn chicks from 0 to 4 weeks of age. A purified diet was used in the first part of their experiment. A practical-type diet was used in the second part of the experiment to determine biological value of phosphates which could not be evaluated with a purified diet due to the high mortality resulting from low availability of the respective compounds. These phosphate sources were compared to beta-tricalcium phosphate as a standard. A modification of slope ratio bioassay (Finney, 1978) was used to determine the biological value of the phosphates under investigation. Willgeroth *et al*. (1944) reported a greater range in bone ash as a response to vitamin D supplementation in poults than in chicks.

Toe ash

Baird and MacMillan (1942) introduced toe ash measurement as an alternative method for vitamin D bioassay. Toe samples were collected by severing the middle and inside toes of each chick at the middle joint. Composite groups of middle and inside toes were extracted and ashed, and the percent toe ash was calculated on a fat-free, dry basis. Toe ash was compared with the respective tibia ash and was found to be a satisfactory alternative method for vitamin D assay. The composite toe ash percentages showed slightly less deviation than the composite ash percentages of tibiae from the same group of chicks. The middle toes were found to yield more uniform ash contents than the inside toes. They suggested that the use of toe ash rather than tibia ash could eliminate the labor and time involved in removing the tibiae from the chicks and in cleaning them of all adhering flesh. Also it could eliminate the human error in removing inadequate amounts of flesh from some of the tibiae. Toe ash measurements also make it possible to carry out the test without sacrificing the chicks. This allows for several composite samples from a given assay group over time to be assayed to monitor the course of calcification.

Evans and St. John (1944) modified the toe ash procedure and found the ash of unextracted dry toe to yield results similar to those obtained from ash of extracted dry toes and tibia ash. They concluded that the ash of unextracted dry toe can be used as satisfactorily as tibia ash in vitamin D bioassay with the toe being slightly more sensitive than the tibia. The procedure is less time consuming than the tibia ash and makes the sacrifice of the chicks at the conclusion of the assay unnecessary.

The use of toe ash as a measure of calcification in chicks in mineral experiments was investigated (Evans and Carver, 1944). New Hampshire chicks were fed diets containing various levels of calcium and phosphorus up to 6 weeks of age. Toe ash was compared with tibia ash and was found to be a satisfactory measure for use in mineral experiments. They also reported that preliminary work with turkeys indicated that the toe can be satisfactorily removed without adverse effects on the poults.

Fritz and Roberts (1968) confirmed the value of toe ash in vitamin D bioassays. They found the toe ash data to have a smaller coefficient of variation than the tibia ash data. Phosphorus utilization was also studied, and the range of calcification in the tibia and toe samples was found to be greater than that in the vitamin D assay. The graded levels of phosphorus supplementation from monosodium phosphate ranged from 0 to approximately .4% phosphorus in the diet.

More recently, Yoshida and Hoshii (1977) recommended that toe ash measurement be used in phosphorus bioassays instead of tibia ash. They found toe ash measurement to be superior to tibia ash measurement because of the wider range of linearity when toe and tibia ash data were plotted against levels of added phosphorus. Toe ash and tibia ash have been found to be highly correlated (Yoshida and Hoshii, 1983), and toe ash has the advantage of being a very simple and a labor-saving measurement.

Dietary calcium and vitamin D

Vitamin D_3 and calcium were known to have an effect on phosphorus utilization in poultry. Nelson (1967) stated that phosphorus utilization could be affected by vitamin D_3 when the calcium to phosphorus ratio was unbalanced or not at an optimum. Griffith and Young (1967) reported an improvement in body weight gain and bone ash when vitamin D_3 was added to a purified diet low in phosphorus with 20% soybean meal up to 800 ICU/kg of diet. Further increase of vitamin D_3 up to 25,600 ICU/kg of diet did not produce an improvement in gain or bone ash. In diets with adequate calcium and phosphorus, vitamin D_3 level of 500 ICU/kg of diet was found to produce maximum growth and bone ash responses. Soybean meal was found to improve phosphorus utilization when added to purified diets. Griffith (1968) reported that the anti-rachitic factor associated with soybean meal was due to its physical property rather than a chemical nature since the powdering of the soybean meal eliminated any response in soybean meal but not in casein. Motzok et al. (1967) reported a minimum of 900 ICU of vitamin D_3 per kg of diet was required for an optimum 4-week body weight when chicks were fed practical-type diets containing soft phosphate. When dicalcium phosphate dihydrate was added to the basal diet, 450 ICU vitamin D_3 per kg diet was sufficient to obtain maximum growth and bone ash.

Dietary calcium affects phosphorus utilization more as a calcium to phosphorus ratio than an absolute calcium content. Excess dietary calcium depressed growth and bone ash (Chicco et al, 1967; Motzok et al., 1967). Reports on calcium to phosphorus ratios were based on calcium to total phosphorus ratio rather than a more realistic calcium to available phosphorus (or inorganic or non-phytate phosphorus) ratio. The reportedly favorable 2:1 calcium:phosphorus ratio was calculated to be more than 3:1 when available phosphorus was used (Dilworth and Day, 1965; Nelson et al., 1965; Neagle et al., 1968). Lowering the calcium to total phosphorus ratio below 2:1 was found to depress gain and bone ash (Dilworth and Day, 1965; Twining et al., 1965; Neagle et al., 1968).

Nelson et al. (1968) reported that 39% of the total phosphorus content in dehulled soybean meal was in the non-phytate form and 34% of phosphorus in corn was non-phytate. Approximately one-third of the dietary phosphorus in a typical corn-soybean meal diet is in the non-phytate form and is available to the birds.

With low dietary phosphorus (.3% available phosphorus), increasing calcium to available phosphorus ratio from 1.5:1 to 2:1 and 3.5:1 improved body weight gain and bone ash (Chicco et al., 1967). With adequate dietary phosphorus, calcium to available phosphorus ratios higher than 2:1 were not investigated, and growth and bone ash responses were found to be decreasing with the narrowing of the Ca:P from 2:1 to .9:1. Neagle et al. (1968) found the calcium to available phosphorus ratio of 3.5:1 to produce a higher gain and toe ash than the ratio of 1.8:1. Dilworth and Day (1965) reported that with .22 and .42% available phosphorus in the diet, the optimum calcium to available phosphorus ratios were 3.16:1 and 2.37:1, respectively. At .42% available phosphorus, gain and bone ash were not decreased when calcium to available phosphorus ratio was increased to 3.17:1.

Griffith and Young (1967) reported that the depression effects when dietary calcium level was increased to over 1.3% up to 2.2% were markedly reduced when 20% soybean meal was added to the purified diet. The increase of calcium to phosphorus ratio of 3:1 to 5:1 did not produce a significant decrease in bone ash content when phosphorus was supplemented as dicalcium phosphate dihydrate. Nelson et al. (1965) reported that the depressing effect of excess calcium was most prominent with the anhydrous dicalcium phosphate and beta-tricalcium phosphate.

Availability of calcium from various calcium phosphate sources may also play a role in phosphorus utilization studies. Damron et al. (1967) reported that maximum growth response was not possible at any level of phosphorus and calcium supplementation with soft phosphate. Supplementation of between .1 and .2% calcium from ground limestone, however, improved the tibia ash to the positive control level. Motzok et al. (1967) found that at least .15 to .20% dietary calcium must be supplied as $CaCO_3$ to provide maximum growth and bone ash. Damron and Harms (1968) reported that a wider calcium to phosphorus ratio was required for soft phosphate at a given dietary phosphorus level than for defluorinated phosphate or calcium phosphate. Motzok et al. (1967) reported the availability of calcium from soft phosphate to be 70% as available as calcium from limestone or dicalcium phosphate.

Both fixed level of calcium and fixed calcium to phosphorus ratio were employed in phosphorus bioassay. Waldroup _et al_. (1965a) used a fixed calcium level of .6% to study the bioavailability of plant phosphates. Fixed level of dietary calcium was studied in a phosphorus bioassay (Waldroup _et al_., 1965b). A dietary calcium level of 1% was compared with a total calcium to total phosphorus ratio of 2:1 and a "sliding" ratio of total calcium to total phosphorus from 1.2:1 at .05% added phosphorus to 1.8:1 at .30% added phosphorus which somewhat mimics a constant calcium to available phosphorus ratio. Relative bioavailability of soft phosphate with a commercial dicalcium phosphate as a standard was higher with the constant calcium level than with either ratio regimen. The two ratios yielded the same bioavailability for the soft phosphate. This could be explained by the improved growth and bone ash responses at low phosphorus levels with the fixed and the sliding ratios. The responses at the highest dietary phosphorus levels were the same for all treatments which made the ratio of the slopes less prominent, thus, the less difference between the two phosphate sources with the constant calcium level.

The depressing effects of high calcium level at low dietary phosphorus may have less impact on phosphorus utilization when an asymptotic regression is applied to the growth response in the phosphorus bioassay. The relative bioavailability of the calcium in the phosphate sources which makes up the majority of the dietary calcium in the high phosphorus diets may play a more important role than the high level of dietary calcium. The calcium in defluorinated phosphate was found to be about equal to that in calcium phosphate and more available than that in soft phosphate (Damron and Harms, 1968). The calcium in defluorinated was not compared directly to the calcium in dicalcium but appeared to be equal or nearly equal based on the estimated 70% relative availability of calcium from soft phosphate as compared with limestone or dicalcium phosphate (Motzok et al., 1967).

Standard phosphate

Ideally, phosphorus in a phosphate compound to be used as a standard in a phosphorus bioassay should be completely available to the test animal. In practice, the standard phosphate should have the highest phosphorus availability possible. Relative bioavailability value for test phosphate higher than 100% is not meaningful since available phosphorus content in a phosphate compound cannot be higher than its total phosphorus content.

Beta-tricalcium phosphate has been commonly used as a standard in a phosphorus bioassay which resulted in phosphorus in nearly all commercial phosphate products being better than 100% available. The bioavailability of phosphorus in the beta-tricalcium phosphate has consistently been shown to be inferior to phosphorus in the commercial monocalcium phosphate, and about equivalent to phosphorus in the commercial defluorinated phosphate (McGillivray, 1978). Dicalcium phosphate dihydrate ($CaHPO_4 2H_2O$) was used as a standard in various studies (Crowley et al., 1963; Motzok et al., 1965; Griffith et al.,1966; Griffith and Young, 1967; Motzok et al., 1967; Motzok, 1968) and was found to be about 25% more available than the anhydrous form.

Rucker et al. (1968) found phosphorus in dicalcium phosphate dihydrate to be 25 to 50% more available than that in the anhydrous form

($CaHPO_4$) in 1-week-old chickens and turkeys. They also found the difference in calcium and phosphorus incorporation into the femur from the hydrous and anhydrous form to decrease gradually with age. For chickens, the difference between the hydrous and anhydrous forms could not be detected at the end of the 5-week experiment. For turkeys, the difference still existed at 5 weeks of age. The availability of phosphorus in the two forms of dicalcium phosphate was correlated with the rate of solution in .1 M acetate buffer. The difference in incorporation of Ca and P was attributed to the difference in solubility of the two forms combined with the rapid rate of passage of feed through the digestive tract of the young birds. Dicalcium phosphate dihydrate, when fed at a low level, was found to be affected less by excess dietary calcium content than the anhydrous form (Griffith and Young, 1967).

Waibel et al. (1984) used monocalcium phosphate monohydrate as a point of reference for comparing results from various experiments. In poults from 0 to 2 weeks of age, they found phosphorus in the monocalcium phosphate monohydrate to be more available than that in the commercial phosphate products with phosphorus in the commercial mono/dicalcium phosphate having the highest bioavailability among the commercial products.

McGillivray (1978) summarized the biological values of phosphate compounds from various reports. Phosphorus in monocalcium phosphate monohydrate was found to have a biological value in the range of 120 to 135%, and phosphorus in dicalcium phosphate dihydrate was reported to have a biological value of 120% when phosphorus in beta-tricalcium phosphate was set at 100%. The choice for a standard phosphate, therefore, lies between monocalcium phosphate monohydrate and dicalcium phosphate dihydrate, both having been shown consistently to have a higher biological value than the commercial phosphate products.

Analysis

Slope ratio bioassay (Finney, 1978) has been the analysis of choice for determining biological values of various feed ingredients. The simple linear regression equation:

$$Y = \beta_0 + \beta_1 X$$

can be applied to a response Y to dose X, in many cases, the response Y being body weight gain. The multiple linear regression equation:

$$Y = \beta_0 + \beta_1 X_1 + \ldots + \beta_n X_n$$

is used for the dose responses of two or more feed ingredients each of which makes up a series of diets based on the same basal diet. The intercept, β_0, is the response at the zero dose and is considered to be the same for all ingredients under test. Therefore, the ratio of the slopes, β_n/β_1, represents the relative bioavailability of ingredient X_n to ingredient X_1.

The main condition that must be met is that the dose response be linear. Any nonlinear portion of the collected data must be discarded. The response lines must be linear to the zero dose level. Any curvature in the response lines indicates statistical invalidity. Thus, it is not uncommon to add a small amount of standard compound to the basal diet to bring the conditional zero dose response up to the linear portion of the curves, or the responses at the zero dose not used in the regression calculation (Finney, 1978). The slope ratio bioassay assumes that data outside the linear range are irrelevant to the final calculation and must be omitted in order not to violate statistical validity of the test.

Nelson and Peeler (1961) applied parallel line assay to determine phosphorus bioavailability. Parallel line assay involves a transformation of the data, in most cases a logarithmic transformation, but is still restricted to within the range of linearity after the transformation. Again, intersection of the true regression lines at a zero dose is required for statistical validity. Problems arise in biological systems since growth curves are generally nonlinear with sigmoidal and asymptotic curves being the main responses. Transformed data still exhibit nonlinearity in most cases and portions of the data must be discarded.

Parks (1970) applied the law of diminishing return to body weight and feed consumption for the growth curve:

$$W - w = (A - w)(1 - e^{-BF})$$

where:

W = body weight,

w = initial body weight,

A = mature body weight,

F = cumulative feed consumed, and

.693/B = feed required for a growth increment of (A-W)/2.

The equation can be rewritten as:

$$Y = \beta_0 + \beta_2(1 - e^{-\beta_1 X})$$

where:

Y = body weight,

X = level of test ingredient,

β_0 = response Y at zero dose X,

$\beta_0 + \beta_2$ = upper asymptote, and

β_1 = response coefficient for dose X.

Robbins et al. (1979) described some dose-response relationships as being sigmoidal with an equation:

$$Y = \beta_0 + \frac{\beta_3}{(1 + e^{\beta_2 - \beta_1 X})}$$

where:

$\beta_0 + \beta_3/(1+e^{\beta_2})$ = a lower asymptote, and

$\beta_0 + \beta_3$ = an upper asymptote.

Other responses that are not evidently sigmoidal follow an asymptotic equation:

$$Y = \beta_0 + \beta_2(1 - e^{-\beta_1 X})$$

which was reported by Park (1970). Robbins et al. (1979) stated that the asymptotic response noted was probably the high-dose portion of the sigmoidal response for which the lower doses were not tested or were not attainable.

Noll *et al*. (1984) found that the body weight gain response of poults from 1 to 4 weeks of age to level of added methionine in the diet followed an asymptotic function. They introduced the use of the asymptotic regression as an alternative to the slope ratio bioassay for determining relative bioavailability of feed ingredients.

Sullivan (1966) questioned the use of bone mineralization determinations as the only or the primary assay parameter in phosphorus bioassays. He argued that, since birds are sold on a body weight and grade basis, the primary criterion forming the basis for the biological value of phosphorus should be body weight data. Growth data and bone ash data were included in equations for calculating relative bioavailability of phosphorus with the major contributor to the equation being body weight gain (55% from body weight gain and 5% from gain:feed ratio) and the rest being percent bone ash (40%). The equations given for the bioavailability calculation, however, did not use body weight gain. The two equations are:

$$BV = \frac{(\text{4-wk BW, g})}{10} + \text{4-wk \% bone ash} + (10 \times \text{4-wk FE})$$

$$RBV = \frac{\text{BV of unknown source}}{\text{BV of standard}} \times 100$$

where:

BV = biological value,

BW = body weight,

FE = feed efficiency (gain:feed ratio), and

RBV = relative biological value.

The arbitrary assignment of degree of contribution by various measurements makes the triple response method of questionable value. The triple response method made possible the bioassay of phosphorus using only one level of supplementation. A simple calculation can be made to confirm or dispute the validity of the triple response method. Using the data from the first dicalcium phosphate in the first experiment, the biological values for .20 and .35% added phosphorus levels are 104.9 and 115, respectively. Thus, the calculated relative biological value for phosphorus in the first dicalcium phosphate added at .20% as compared with phosphorus from the same source added at .35% was 91%. If the triple response method gives an accurate measure of relative bioavailability, the calculated relative bioavailability should be 57% ($.20 \div .35 \times 100$). There was no provision for confidence limit calculations in the triple response method. Nelson (1967), in his review of phytate phosphorus utilization by poultry, noted that body weight parameters were less sensitive and less accurate than the percent bone ash in determining phosphorus bioavailability.

SERIES I. BIOLOGICAL VALUES OF EXPERIMENTAL PHOSPHATES AS COMPARED WITH VARIOUS COMMERCIAL PHOSPHATE SOURCES

Two experiments were conducted to determine the relative bioavailability of phosphorus in four samples of an experimental defluorinated phosphate product as compared with three samples each of commercial mono/dicalcium phosphate, commercial dicalcium phosphate, and commercial defluorinated phosphate. A purified-grade dicalcium phosphate dihydrate was used as the standard. The experimental defluorinated phosphate samples were prepared from rock collected from different locations within a quarry and were proposed for commercial use.

Waibel et al. (1984) supplied a minimum of 0% added inorganic phosphorus and a maximum of .56% added phosphorus in their phosphorus bioassay using poults from 0 to 2 weeks of age. The growth and bone ash data showed an asymptotic response within the range of supplementation. Phosphorus supplementations employed in Experiments 1 and 2 ranged from .09% to .45% added inorganic phosphorus with the calculated organic phosphorus level in the basal diet of .44%. The maximum added phosphorus level supplied the poults with a level of total available phosphorus at the NRC (1984) requirement for poults from 0 to 4 weeks of age.

MATERIALS AND METHODS

For each of two experiments, 540 day-old male Large White poults, obtained from a commercial hatchery, were randomized into 60 pens of Petersime batteries. Poults were group-weighed at 1 day of age and at weekly intervals to 3 weeks of age. Feed and mortality records were kept so that average feed consumption and average feed efficiency for the turkeys in each pen could be determined on a weekly basis.

The composition of the basal diet, which contained .44% organic phosphorus, is presented in Table 1.1, and the compositions of the 14 sources of phosphorus are presented in Table 1.2. Each phosphorus source was added to the basal diet to supply .09, .18, .27, and .45% inorganic phosphorus to form 56 diets. Phosphorus supplementations were made in place of ground limestone, salt, and ground yellow corn to maintain constant levels of calcium and sodium in the diets. Each of the diets was fed to one pen of poults in each experiment, except those which contained dicalcium phosphate (dihydrate, purified), each of which was fed to two pens.

The standard dicalcium phosphate,[1] $CaHPO_4 2H_2O$, was assumed to contain 18.0% phosphorus at time of diet preparation. However, subsequent analysis revealed that it contained 24.07% calcium and 18.6% phosphorus as a result of apparent loss of some water of hydration. Levels of .093,

---

[1] A Fisher Scientific Company product (dated 10/2/1968) designated as "calcium phosphate, dibasic - secondary, purified" (C-123) with the chemical formula of $CaHPO_4 2H_2O$.

.186, .279, and .465% phosphorus were therefore supplied to the diets by the standard, and calculations were made accordingly.

At 3 weeks of age, the left middle toes of all poults within a pen were collected and ashed as a group composite (A.O.A.C., 1984). The toes were excised at the second joint from the distal end. Entire toes were pooled by pens, cleaned, dried at 105 C for 18 hours, allowed to cool down to room temperature in a desiccator, weighed, and ashed at 600 C for 4 hours. Measurements of average body weight and toe ash as percentage of dry toe were determined for each pen and subjected to analyses of variance and nonlinear regression analyses (Noll _et al_., 1984). The nonlinear (asymptotic) model:

$$Y = \beta_0 + \beta_2(1 - e^{-\beta_1 X})$$

where:

Y = body weight or toe ash percentage,

X = level of added inorganic phosphorus,

β_0 = response Y at zero dose X,

$\beta_0 + \beta_2$ = upper asymptote, and

β_1 = response coefficient for dose X,

was used in the NLIN procedure (SAS Institute Inc., 1985). Nonlinear least squares regression calculations were based on the DUD method of Ralston and Jennrich (1978). Relative bioavailability was calculated by the multiple regression model:

$$Y = \beta_0 + \beta_{n+1}(1 - e^{-\beta_1 X_1 - \cdots - \beta_n X_n})$$

where:

Y = body weight or toe ash percentage and

X_n = level of added inorganic phosphorus from source n,

and the ratio of β_n to β_1 is the relative bioavailability of X_n to X_1. The 95% confidence limit calculation is presented in Appendix A.

RESULTS

During the 3-week experiment, 65, 19, 5, and 3% of the poults died from diets containing .09, .18, .27, and .45% added phosphorus, respectively (Table 1.3). Mortality was 31% of the poults fed diets containing the lowest level of added phosphorus from $CaHPO_4 2H_2O$ in contrast to 65 to 80% of those fed diets containing the other phosphates. In addition, 29 and 41% of the poults died when fed .18% added phosphorus from the experimental and commercial defluorinated phosphates, respectively, in contrast to 4 and 9% of the poults fed diets containing the same level of phosphorus from the mono/dicalcium and dicalcium phosphates, respectively. Thus, the phosphorus in the standard appeared more available than that in the other phosphates, and the phosphorus in the mono/dicalcium and dicalcium phosphates appeared more available than that in the defluorinated phosphates.

Average feed consumption and average feed efficiency data could not be calculated accurately due to high mortality among birds fed low phosphorus diets and was not included in the bioavailability analyses. Average body weights and the measurements of percent ash of the dry toe of the turkeys in each pen of the two experiments are presented in Tables 1.4 and 1.5, respectively. From feeding diets containing .09, .18, .27, and .45% added phosphorus, the average 3-week body weights were 210, 314, 381, and 416 g, respectively, in Experiment 1 and 230, 353, 415, and 447 g, respectively, in Experiment 2. Percentages of ash of the dry toe of turkeys fed diets containing .09, .18, .27, and .45% added phosphorus were

6.64, 9.95, 13.38, and 15.25%, respectively, in Experiment 1 and 6.55, 9.84, 12.94, and 14.30%, respectively, in Experiment 2.

In general, the body weights or the measurements of percent ash of the dry toes of the turkeys were an asymptotic function of the levels of phosphorus added to the diets as shown in Figures 1.1 through 1.4. In these plots, phosphorus sources were pooled into the following groups: dicalcium phosphate (dihydrate, purified), mono/dicalcium phosphates (21% P), dicalcium phosphates (18.5% P), commercial defluorinated phosphates, and experimental defluorinated phosphates. Differences in biological values of the phosphorus in the various sources exist as illustrated in these figures.

The relative biological values of the various phosphorus sources when compared with the dicalcium phosphate (dihydrate, purified) standard are presented in Table 1.6. The average biological values of phosphorus in all sources using body weight and toe ash measurements were 75.8 and 81.8%, respectively, in Experiment 1 and 79.5 and 80.5%, respectively, in Experiment 2. The lower average biological values obtained from the body weight data in the first experiment are thought to be due to a relatively high value for the standard and are probably within the range of normal variation. The four determinations of relative biological value of phosphorus in a given sample obtained by using body weight or toe ash measurements in the two experiments were not significantly different (Table 1.6).

Phosphorus in the mono/dicalcium phosphates was 92.6% as available as that in the standard. This value was significantly greater than the 69.6% value for the commercial defluorinated phosphates, the 75.5% value for the experimental defluorinated phosphates, and the 81.2% value for the dicalcium phosphates. The phosphorus in the dicalcium phosphates was significantly more available than that in the commercial defluorinated phosphates. The average biological value of phosphorus in the experimental defluorinated phosphates was intermediate to that of the dicalcium phosphates and of the commercial defluorinated phosphates. A difference of about 5% between the average biological values of three vs. three or four samples was required for significance in this study (Table 1.6). Differences among the individual sources of phosphorus within a group as presented in Table 1.6 were not significant based on Duncan's multiple range test. The least significant difference required between any two samples was 8%. The level of cadmium (0 to 26 ppm) in the various commercial and experimental phosphates was not related to the biological availability of the phosphorus in this study.

DISCUSSION

Evidence from this study indicates that differences exist among biological values of phosphorus from different types of commercial phosphates. With the biological value of phosphorus in the commercial mono/dicalcium phosphates (21% P) set at 100%, the biological values of phosphorus in the dicalcium phosphates (18.5% P) and the commercial defluorinated phosphates (18% P) averaged 88 and 75%, respectively. Phosphorus in commercial defluorinated phosphates were only 86% as biologically effective as that in the dicalcium phosphates. Waibel et al. (1984) reported similar results in poults from 0 to 2 weeks of age. With the biological value of 7 mono/dicalcium phosphates (21% P) set at 100%, they reported that the average biological values of 20 dicalcium phosphates (18.5% P) and of 20 defluorinated phosphates were 93 and 81%, respectively. In their study, phosphorus in the defluorinated phosphates was only 87% as biologically effective as that in the dicalcium phosphates. Data from other studies (Nelson and Walker, 1964; Sullivan, 1966; Pensack, 1974; McGillivray, 1978) with a limited number of phosphates tend to support these relative biological values.

A difference of about 15% in biological value was required to detect a significant difference between two samples of phosphorus using either body weight or toe ash measurements in one experiment (Table 1.6). Nelson and Walker (1964) reported a standard deviation about the biological value of a phosphate of ±5%, thus requiring a difference of 14% for significance between phosphorus samples when bone ash was used as a measurement in one experiment of their studies (difference required for significance =

st$\sqrt{2}$ where s = standard deviation). They also reported that about six times as many pens of birds were required to secure the same degree of accuracy if body weight rather than bone ash was used as the criterion of measurement. More recently, Yoshida and Hoshii (1979) and Huyghebaert et al. (1980) reported differences of 10 to 25% and 25 to 37%, respectively, were required between the biological values of samples of phosphorus to be significant in their studies. When all data in this study were pooled, a difference of 8% in biological value between samples and a difference of 5% in biological value between averages of groups of three samples vs. three or four samples were significantly different ($P<.05$). Thus, the results of this study provided an equally or more sensitive bioassay than those of other researchers.

The slope ratio bioassay (Finney, 1978) has been the analysis of choice for determining the relative biological value of different phosphorus sources (Hurwitz, 1964; Waibel et al., 1984). However, a plot of either body weight, bone ash, or toe ash on level of added phosphorus results in asymptotic curves rather than linear lines. Thus, the slope ratio bioassay is not applicable to data with curvature, and the application of the asymptotic curve as a new approach to a phosphorus bioassay was implemented in this study.

Dicalcium phosphate dihydrate was used as a standard in this study. Other researchers have also used this compound as a standard (Wilcox et al., 1955; Hurwitz, 1964) or have used reagent grade mono/dicalcium phosphate monohydrate (Motzok et al., 1956; Waibel et al., 1984). These hydrated compounds are highly utilized and provide a better standard for attainment than beta-tricalcium phosphate.

The labor involved in measuring phosphorus bioavailability values is much less when toe ash rather than bone ash is a criterion. In this study, the results from using toe ash as a measurement of response were as sensitive as those from using body weight.

SUMMARY

Two experiments, each utilizing 540 male turkey poults, were conducted to determine the biological value of phosphorus from 13 different sources when compared with that from dicalcium phosphate dihydrate as a standard. Each phosphorus source was added to a basal diet composed of 40.4% ground yellow corn and 52% soybean meal to supply .09, .18, .27, or .45% inorganic phosphorus. Each of 60 diets, which included duplicates of the standard source, was fed to a pen of nine male poults from 1 day to 3 weeks of age in each experiment. Body weight and toe ash measurements of poults collected at 3 weeks of age were used in nonlinear (asymptotic) bioassays to obtain relative biological values of phosphorus from various sources.

With the dicalcium phosphate standard set at 100%, the average relative biological values of three samples from each of commercial mono/dicalcium phosphate (21% P), dicalcium phosphate (18.5% P), and defluorinated phosphate (18% P) were 93, 81, and 70%, respectively. Four samples of experimental defluorinated phosphate averaged 76%. Differences of about 5% between values were required for statistical significance, indicating significant differences among these biological values of phosphorus from the various phosphate sources in diets of young turkeys.

TABLE 1.1. Composition of the basal diet[1] (Series I and III)

Ingredient	Amount
	(g/kg)
Ground yellow corn	404.4
Stabilized fat	30.0
Dehulled soybean meal	520.0
Ground limestone	35.0
Iodized salt	5.0
Trace mineral mix[2]	.7
Vitamins and feed additives[3]	2.9
DL-Methionine	2.0
Total	1000.0

[1]Contained by calculation using values from National Research Council (1984): 1.48% calcium and .44% total phosphorus.

[2]Supplied per kilogram of diet: 150 mg manganese, 100 mg zinc, 70 mg iron, 10 mg copper, and 1 mg iodine from manganese oxide, zinc oxide, ferrous sulfate heptahydrate, anhydrous cupric sulfate, and potassium iodate, respectively.

[3]Supplied per kilogram of diet: 6,600 IU vitamin A, 3,300 ICU vitamin D_3, 11 IU vitamin E, 7 mg menadione sodium bisulfite, 1.1 mg thiamin HCl, 5.5 mg riboflavin, 16.5 mg D-calcium pantothenate, 66 mg niacin, 750 mg choline chloride, .015 mg vitamin B_{12}, 1.1 mg folic acid, .22 mg biotin, 2.2 mg pyridoxine HCl, 125 mg ethoxyquin, .2 mg selenium from sodium selenite, and 44 mg bacitracin from zinc bacitracin.

TABLE 1.2. Composition of phosphorus sources[1]

Source of phosphorus	Calcium	Phosphorus	Sodium	Cadmium
	------------(%)------------			(ppm)
Standard				
$CaHPO_4 \cdot 2H_2O$	24.07	18.60	...[2]	...
Mono/dicalcium phosphate[3]				
#1	17.96	20.52	...	26
#2	15.44	20.49	...	7
#3	15.53	20.78	...	3
Dicalcium phosphate[3]				
#1	22.96	18.83	...	23
#2	20.32	18.45	...	5
#3	20.46	17.68	...	3
Defluorinated phosphate[3]				
#1	30.48	18.11	4.90	0
#2	31.99	18.15	5.46	2
#3	30.34	18.26	4.28	1
Defluorinated phosphate (E)[4]				
#1	31.78	18.52	4.33	8
#2	31.78	18.63	4.63	7
#3	32.16	18.60	4.70	6
#4	31.42	18.77	5.03	10

[1]All values were derived from chemical analyses (Association of Official Analytical Chemists, 1984) of subsamples of these products: phosphorus by the Automated Method (2.032-2.039), calcium by the Dry Ash Method (7.101), and cadmium by the Atomic Absorption Spectrophotometric Method (33.089-33.094).

[2]Values not determined and assumed to be negligible.

[3]Commercial sources.

[4]Experimental sources (samples of product proposed for commercial use).

TABLE 1.3. Mortality during the 3-week experiment[1]

Source of phosphorus	Experiment 1 Added phosphorus, %				Experiment 2 Added phosphorus, %			
	.09	.18	.27	.45	.09	.18	.27	.45
Standard								
$CaHPO_4 \cdot 2H_2O$	3	0	0	0	2	0	0	1
$CaHPO_4 \cdot 2H_2O$	3	0	0	0	3	0	0	0
Mono/dicalcium phosphate								
#1	5	0	0	0	4	0	0	1
#2	8	0	0	0	6	0	0	1
#3	5	2	0	0	6	0	1	0
Dicalcium phosphate								
#1	7	2	0	1	6	2	1	0
#2	6	1	0	0	9	0	0	0
#3	8	0	2	1	7	0	0	2
Defluorinated phosphate								
#1	8	4	2	0	4	2	1	0
#2	5	2	0	0	6	4	2	0
#3	7	4	0	1	6	6	0	0
Defluorinated phosphate (E)								
#1	7	3	1	0	8	3	0	0
#2	6	3	1	0	5	4	0	0
#3	7	1	0	0	4	0	0	0
#4	8	5	1	0	7	0	1	0
Total	93	27	8	3	83	21	6	5

[1]Number of deaths from nine poults per pen.

TABLE 1.4. Average body weight of poults at 3 weeks of age

Source of phosphorus	Experiment 1 Added phosphorus, %				Experiment 2 Added phosphorus, %			
	.09	.18	.27	.45	.09	.18	.27	.45
	(g)							
Standard								
$CaHPO_4 \cdot 2H_2O$	241	343	404	432	251	415	429	471
$CaHPO_4 \cdot 2H_2O$	241	387	424	422	274	394	421	496
Mono/dicalcium phosphate								
#1	206	351	416	418	254	398	418	442
#2	206	325	422	431	250	354	401	448
#3	231	300	406	446	253	356	459	459
Dicalcium phosphate								
#1	180	316	337	461	218	339	434	470
#2	211	303	400	427	...[1]	312	423	446
#3	238	307	413	422	232	362	415	385
Defluorinated phosphate								
#1	153	290	324	420	230	327	386	437
#2	210	271	338	377	223	309	409	426
#3	157	314	371	388	208	340	409	448
Defluorinated phosphate (E)								
#1	236	317	384	385	170	350	428	454
#2	203	314	357	421	231	366	366	449
#3	202	306	338	397	223	343	411	430
#4	243	272	381	385	209	322	410	457
Average	210	314	381	416	230	353	415	447

[1]All poults died.

TABLE 1.5. Average ash content of dry toes from poults at 3 weeks of age

Source of phosphorus	Experiment 1 Added phosphorus, % .09	.18	.27	.45	Experiment 2 Added phosphorus, % .09	.18	.27	.45
	(%)							
Standard								
$CaHPO_4 \cdot 2H_2O$	7.27	11.51	15.26	15.72	7.74	11.78	13.68	14.64
$CaHPO_4 \cdot 2H_2O$	6.14	12.10	14.55	16.10	7.24	11.18	14.02	14.36
Mono/dicalcium phosphate								
#1	6.49	11.03	13.84	15.33	6.27	12.17	13.17	14.25
#2	7.16	11.50	14.75	14.50	7.02	10.36	14.16	15.21
#3	6.64	11.29	14.78	14.84	6.18	11.42	13.77	14.96
Dicalcium phosphate								
#1	7.61	8.69	13.78	14.96	7.01	10.43	13.19	13.86
#2	5.56	8.77	14.03	15.60	...[1]	9.10	13.20	14.19
#3	5.36	10.83	13.97	15.13	6.19	10.05	14.05	15.04
Defluorinated phosphate								
#1	7.18	8.66	12.43	15.33	7.19	8.78	10.83	14.51
#2	5.64	9.12	10.36	14.99	5.22	8.53	12.51	14.04
#3	8.24	8.78	13.01	15.15	5.90	8.57	10.77	13.34
Defluorinated phosphate (E)								
#1	6.39	9.11	11.99	15.68	7.42	8.63	12.70	13.88
#2	6.45	9.29	12.23	15.10	6.07	8.98	12.67	13.26
#3	6.15	9.32	13.13	15.27	5.14	8.49	13.72	15.12
#4	7.33	9.25	12.52	15.02	7.10	9.16	11.70	13.78
Average	6.64	9.95	13.38	15.25	6.55	9.84	12.94	14.30

[1]All poults died.

TABLE 1.6. Biological values of various phosphorus sources compared with dicalcium phosphate (dihydrate, purified grade) as measured by body weight and toe ash measurements collected from turkeys at 3 weeks of age

Source of phosphorus	Body weight		Toe ash		
	Exp 1[1]	Exp 2	Exp 1	Exp 2	Average
	(%)				
Mono/dicalcium phosphate					
#1	90.5	97.5	91.1	95.9	93.8
#2	86.9	85.0	99.1	96.3	91.8
#3	85.5	93.9	96.4	95.5	92.8
Average	87.6	92.2	95.5	95.9	92.8
Dicalcium phosphate					
#1	70.8	80.8	82.7	88.5	80.7
#2	79.9	72.0	79.0	78.4	77.3
#3	87.9	82.1	85.6	89.2	86.2
Average	79.5	78.3	82.4	85.4	81.4
Defluorinated phosphate					
#1	59.5	73.9	76.5	71.6	70.4
#2	61.6	72.1	65.3	69.5	67.1
#3	66.8	75.4	82.6	63.3	72.0
Average	62.6	73.8	74.8	68.2	69.8
Defluorinated phosphate (E)					
#1	82.2	73.3	75.1	78.2	77.2
#2	73.9	79.0	75.3	72.8	75.3
#3	67.7	78.0	78.7	76.4	75.2
#4	73.7	73.3	78.9	74.8	75.2
Average	74.4	75.9	77.0	75.6	75.7
Average	75.9	79.7	82.0	80.8	79.6
Difference required for significance (P<.05)					
Between samples[2]	18.2	14.2	15.9	12.6	8.1
Between groups of samples[3]	10.5	8.2	9.2	6.9	4.7

[1]Exp = Experiment.

[2]Between any two samples.

[3]Average of three samples *vs.* three or four samples.

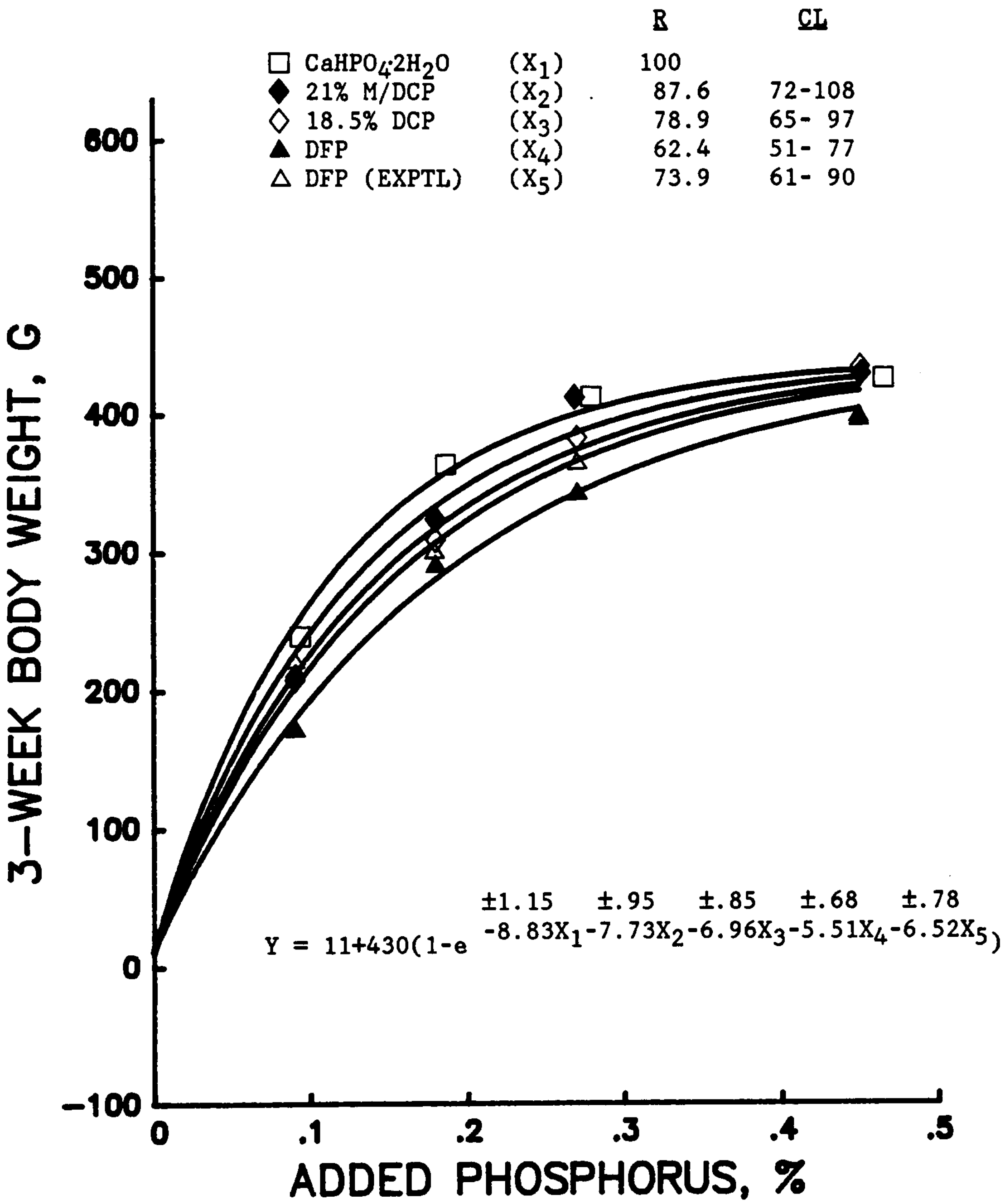

FIGURE 1.1. Plot of body weight of turkeys at 3 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from various sources in Experiment 1. M/DCP=Mono/dicalcium phosphate, DCP=dicalcium phosphate, DFP=defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from experimental sources.

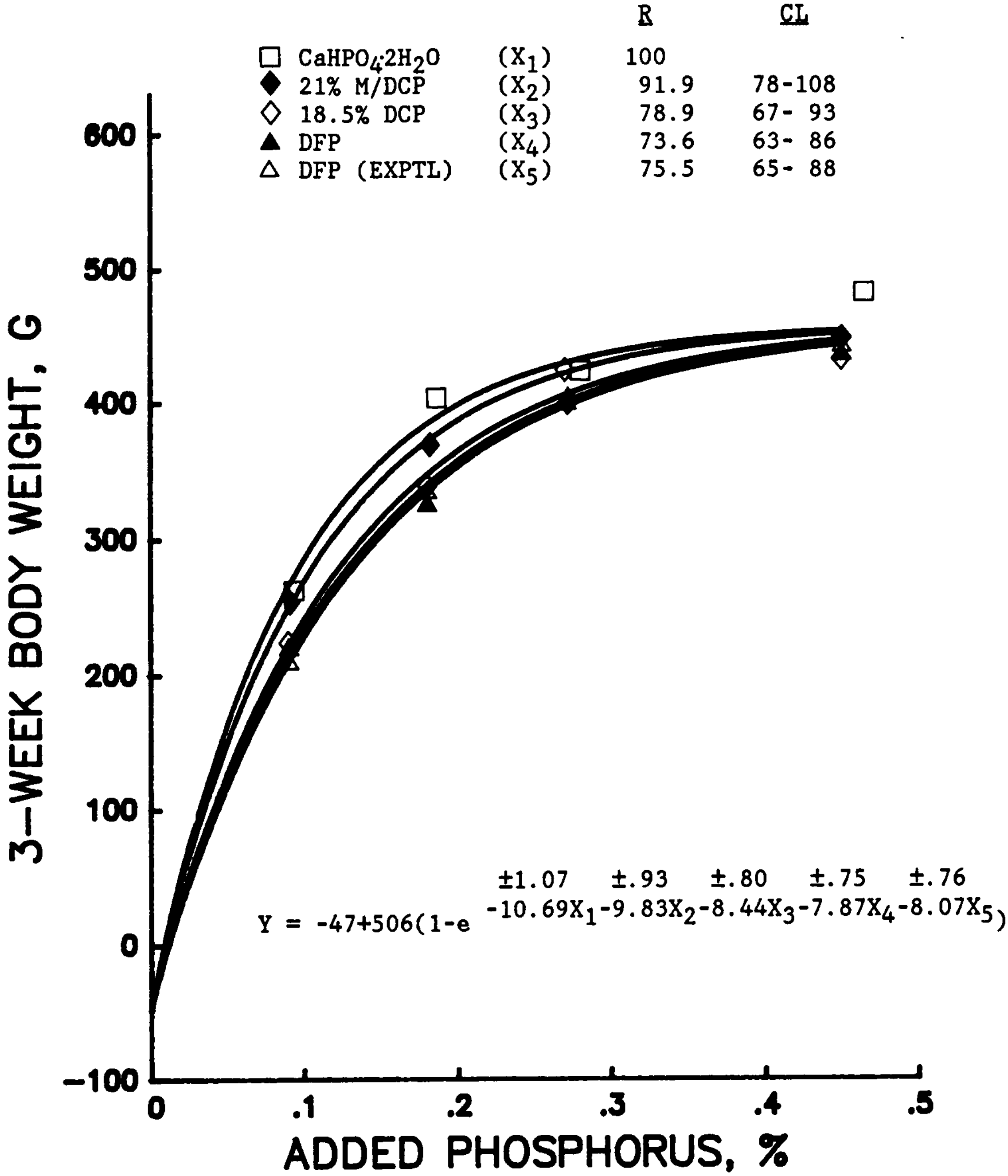

FIGURE 1.2. Plot of body weight of turkeys at 3 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from various sources in Experiment 2. M/DCP=Mono/dicalcium phosphate, DCP=dicalcium phosphate, DFP=defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from experimental sources.

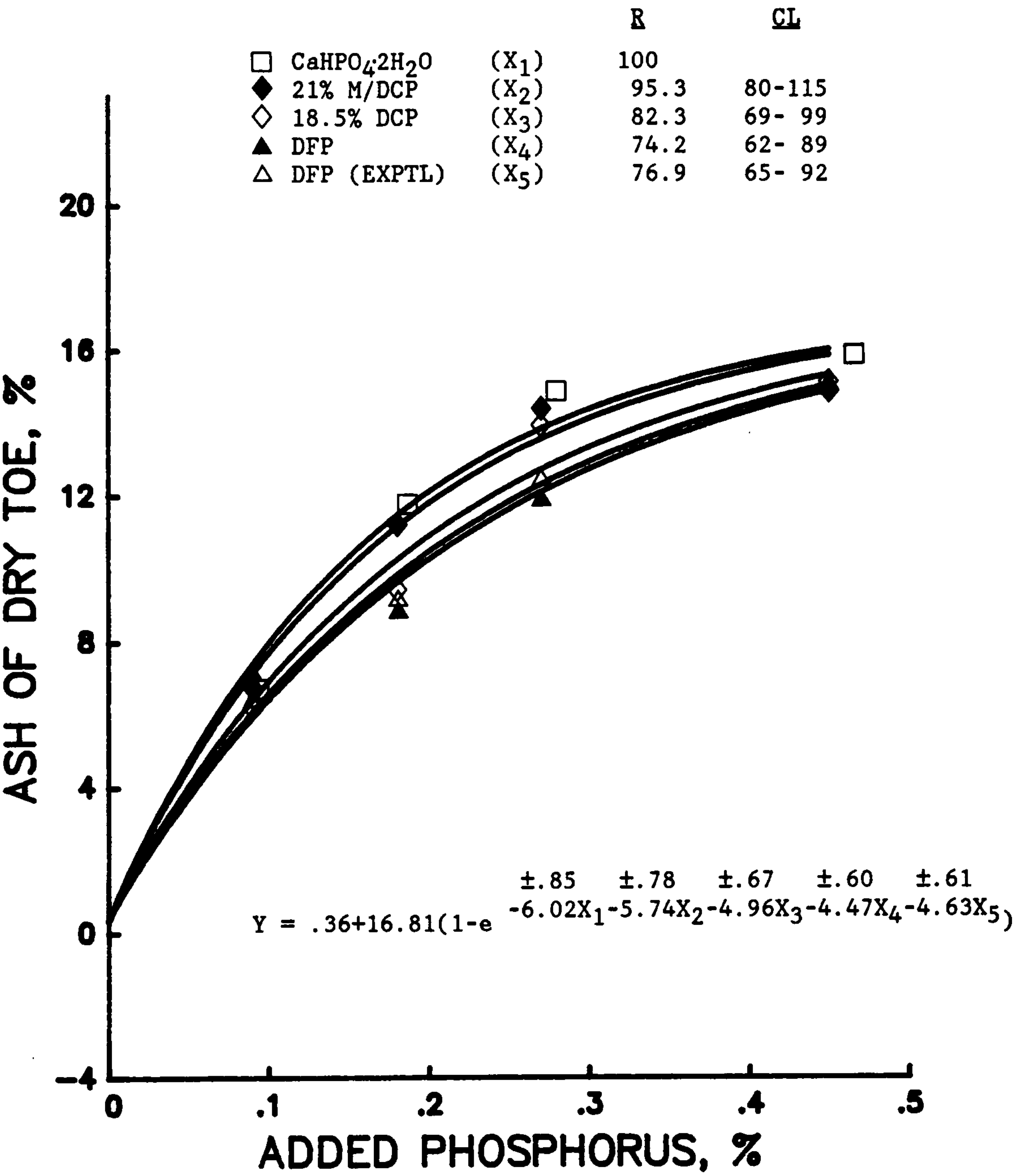

FIGURE 1.3. Plot of toe ash contents of turkeys at 3 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from various sources in Experiment 1. M/DCP=Mono/dicalcium phosphate, DCP=dicalcium phosphate, DFP=defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from experimental sources.

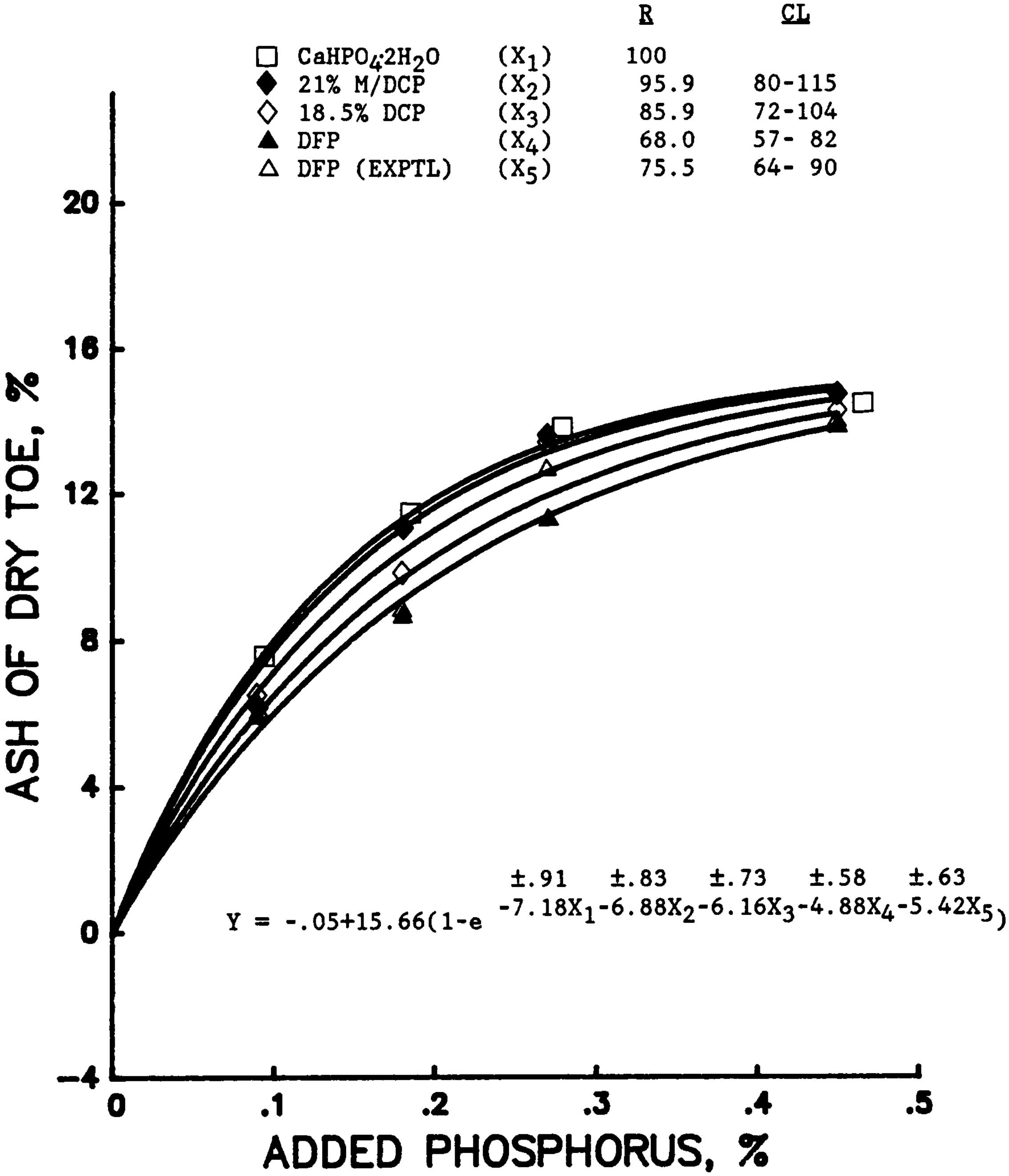

FIGURE 1.4. Plot of toe ash content of turkeys at 3 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from various sources in Experiment 2. M/DCP=Mono/dicalcium phosphate, DCP=dicalcium phosphate, DFP=defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from experimental sources.

SERIES II. EFFECTS OF PRE-EXPERIMENTAL PHOSPHORUS FEEDING ON SUBSEQUENT RELATIVE BIOAVAILABILITY DETERMINATION OF PHOSPHORUS FROM TWO COMMERCIAL SOURCES

In the two experiments in Series I, phosphorus in a commercial dicalcium phosphate (dicalcium phosphate #2) and phosphorus in a commercial defluorinated phosphate (defluorinated phosphate #3) were found to be 77 and 72% as available as phosphorus in the dicalcium phosphate dihydrate, respectively, when 3-week body weight and 3-week toe ash content were used in the asymptotic regression analyses. The difference between biological values of the two products was not significant.

Two experiments were designed to compare directly the bioavailability of a commercial defluorinated phosphate with a commercial dicalcium phosphate in the absence of a standard phosphate. The effect of a pre-experimental diet containing an adequate amount of phosphorus on subsequent experimental diets was examined.

MATERIALS AND METHODS

All poults used in both experiments were hatched at the Turkey Research Center. Sixty-four male and 80 female poults were used in Experiment 1 and 72 male and 72 female poults were used in Experiment 2. Poults were fed a 28%-protein starter diet (Table 2.1) from day one to 1 week of age. Poults of each sex were individually weighed and randomized at 1 week of age into eight pens of a Petersime battery for each experiment. They were group-weighed at 1 week of age and at weekly intervals to 4 weeks of age. Feed consumption and mortality records were kept so that average feed consumption for the turkeys in each pen could be determined on a weekly basis.

Poults were placed on experimental diets at 1 week of age. The composition of the basal diet (Table 2.2) is equivalent to that used in Series I except for the substitution of penicillin in place of bacitracin. One commercial dicalcium phosphate (Dynafos) and one commercial defluorinated phosphate (Multifos) from a different batch from those in Series I were used. Each phosphorus source was added to the basal diet to supply .09, .18, .27, and .45% inorganic phosphorus to form eight diets. Calcium and sodium contents in all experimental diets were held constant as in Series I.

At 4 weeks of age, the left middle and inside toes of all poults were collected by cutting through the joint between the second and third tarsal bones from the distal end. Cleaning of group samples of toes was facilitated by soaking each group of toes in tap water for approximately 1 to 2 minutes to loosen the dirt. The toe samples was then easily cleaned. The group samples of toes were dried, at 105 C for 18 hours, cooled to room temperature in a desiccator, weighed, and ashed for 4 hours at 600 C (Fritz _et al_., 1969). Average body weight gain and toe ash percentage were determined for each pen and subjected to analyses of variance and nonlinear regression analyses (Noll _et al_., 1984).

RESULTS

The mortality of poults from beginning of experiment (1 week of age) to 4 weeks of age was significantly lower than the mortality of poults in Series I due mainly to the feeding of phosphorus-adequate starter diet during the 1-week pre-experimental period (Table 2.3). No differences in mortality were found between the two phosphorus sources (overall mortality of 9 and 8 for Dynafos and Multifos, respectively) and mortality was not related to level of phosphorus fed during the 3-week experimental period. The substitution of penicillin in place of bacitracin may also have an effect on mortality in this experiment.

The average body weight gain from 1 to 3 weeks of age was not significantly different when poults were fed Dynafos or Multifos, with overall averages of 354 and 346 g for Dynafos and Multifos, respectively (Table 2.4). Average body weight gain from 1 to 4 weeks of age began to diverge, with overall averages of 601 and 576 g for poults fed Dynafos and Multifos, respectively (Table 2.5).

Relative bioavailability calculations based on 4-week data were not possible for data from individual experiments and for data from the combined male and female data both within an experiment or with both experiments combined due to the large standard error for the parameters which rendered the slopes to be not significantly different from zero. The only possible relative bioavailability calculations were those based on the combined data of both experiments with male and female analyzed separately. Using data from both experiments, the 1 to 4-week body weight gain as a response to added phosphorus levels follows asymptotic equations:

$$Y = 329.1 + 511.0(1 - e^{-4.42X_1 - 3.73X_2})$$

for male poults, and

$$Y = 351.7 + 361.4(1 - e^{-4.07X_1 - 3.33X_2})$$

for female poults in the combined data from both experiments where Y equals the 1 to 4-week body weight gain and X_1 and X_2 the percent phosphorus added from Dynafos and Multifos, respectively. The relative biological values of Multifos to Dynafos based on 1 to 4-week body weight gain were 84.5 and 81.9% for male and female poults, respectively. However, both numbers were not significantly different from 100% for Dynafos due to the confidence limits of 54-145 and 60-122% for male and female poults, respectively. Plots of body weight gain on added phosphorus levels are presented in Figures 2.1 and 2.2.

The ash contents of dry toes from 4-week-old poults are presented in Table 2.6. In all cases, the ash content of the inside toe was higher than the ash content of the middle toe from the same group of birds. No significant differences were found between the two sexes within each experiment. The inside and middle toes, however, were significantly different and were analyzed separately. The middle toe data did not give a possible solution for a confidence limit calculation when male data from Experiment 1 were used while inside toe data from both sexes and both experiments allowed for relative biological value with confidence limit calculations in all cases. Combining the data from both sexes within each experiment and with middle and inside toes separately yielded similar results, with middle toes from Experiment 1 not allowing confidence limit calculation when data from both sexes were combined (Table 2.7). In all cases but one, biological value of phosphorus in Multifos was not significantly different from phosphorus in Dynafos. Only when inside toe data from females in Experiment 2 were analyzed did Multifos become significantly less available than Dynafos. The asymptotic regression equations for 4-week toe ash when data of both sexes were combined are:

$$Y = 6.57 + 8.61(1 - e^{-2.85X_1 - 2.60X_2})$$

for middle toes from Experiment 1,

$$Y = 6.81 + 8.85(1 - e^{-3.36X_1 - 3.10X_2})$$

for inside toes from Experiment 1,

$$Y = 4.15 + 9.60(1 - e^{-5.01X_1 - 4.17X_2})$$

for middle toes from Experiment 2, and

$$Y = 5.51 + 8.63(1 - e^{-4.99X_1 - 4.24X_2})$$

for inside toes in Experiment 2, where Y equals the 4-week toe ash and X_1 and X_2 the percent phosphorus added from Dynafos and Multifos, respectively. Plots of toe ash data as a response to level of added phosphorus are presented in Figures 2.3 to 2.6.

DISCUSSION

Feeding a starter diet adequate in phosphorus during the first week eliminated the differences in 1 to 3-week body weight gain when poults were fed the two phosphorus sources. The 1 to 4-week body weight gain data began to diverge but the biological values derived from these data were not significantly different. The response to levels of dietary phosphorus followed an asymptotic regression equation for both body weight gain and toe ash data but was much closer to being linear than the results in Series I. Rucker et al. (1968) found the difference in the ability to incorporate phosphorus into femur by poults between hydrated and anhydrous dicalcium phosphate to be diminishing with age. Differences could be detected at 5 weeks of age but the differences were much less than at 1 week of age in poults. The 1-week pre-experimental period when poults were fed a diet adequate in phosphorus was the time when differences in poult's ability to incorporate different phosphorus sources were most prominent. A longer experimental period may be needed if poults were fed a starter diet adequate in phosphorus during the first week after hatch.

In Series I, differences in bioavailability between phosphorus in Multifos and Dynafos were not significant. The relative biological values of phosphorus in Multifos (Defluorinated phosphate #3) to that in Dynafos (Dicalcium phosphate #2) in Series I were 94 and 93% based on body weight and toe ash measurements, respectively. The average relative biological values of phosphorus in Multifos to that in Dynafos in the current experiments were 83 and 88% based on body weight gain and toe ash measurements, respectively. Although significant differences could not be detected, the sample of the commercial defluorinated phosphate was consistently inferior to the sample of the commercial dicalcium phosphate.

By comparing the range of confidence limits calculated from both experiments, inside toes seemed to yield a slightly better precision than middle toes for the bioassay in Experiment 1 while middle toes yielded a slightly better precision than inside toes in Experiment 2 (Table 2.7). Baird and MacMillan (1942) reported that the middle toes yielded more uniform ash contents than inside toes with toe samples being extracted prior to ashing. The current experiments did not confirm the observations of Baird and MacMillan (1942) except for the higher toe ash contents for the inside toes as compared with the middle toes.

SUMMARY

Two experiments were conducted to determine the relative biological value of phosphorus in one commercial dicalcium phosphate to that in one commercial defluorinated phosphate using 144 poults in each experiment. Poults were fed a starter diet sufficient in phosphorus during the first week then put on experimental diets at 1 week of age. Each of the two phosphorus sources was added to the basal diet composed of 40.4% ground yellow corn and 52% dehulled soybean meal to supply .09, .18, .27, or .45% inorganic phosphorus. Each of the 8 diets was fed to a pen of 8 male and a pen of 10 female poults in Experiment 1, and a pen of 9 male and a pen of 9 female poults in Experiment 2 from 1 to 4 weeks of age. Body weight gain from 1 to 4 weeks of age and toe ash measurements at four weeks of age were used in nonlinear (asymptotic) bioassays to obtain relative biological values of phosphorus from one source to the other.

With phosphorus in the commercial dicalcium phosphate set as standard at 100%, the relative biological value of phosphorus in the commercial defluorinated phosphate averaged 88%. The relative biological value of phosphorus in the commercial defluorinated phosphate as compared with that in the commercial dicalcium phosphate was consistently, but not significantly, lower than 100%. The feeding of a starter diet sufficient in phosphorus during the first week post-hatch may have masked any significant differences between the two phosphate products. Overall mortality was significantly lowered by feeding a starter diet.

TABLE 2.1. Composition of the starter diet[1]

Ingredient	Amount
	(g/kg)
Ground yellow corn	463.3
Stabilized fat	10.0
Dehulled soybean meal	424.0
Menhaden fish meal	50.0
Dicalcium phosphate (Dynafos)	28.0
Ground limestone	15.0
Iodized salt	4.0
Trace mineral mix[2]	1.0
Vitamins and feed additives[3]	2.7
DL-Methionine	2.0
Total	1000.0

[1]Contained by calculation using values from National Research Council (1984): 27.8% crude protein, 1.56% calcium, and 1.06% total phosphorus.

[2]Supplied per kilogram of diet: 150 mg manganese, 120 mg zinc, 40 mg iron, 6 mg copper, and 1.5 mg iodine from manganese oxide, zinc oxide, ferrous sulfate, cupric sulfate, and potassium iodate, respectively.

[3]Supplied per kilogram of diet: 13,200 IU vitamin A, 3,300 ICU vitamin D_3, 11 IU vitamin E, 7 mg menadione sodium bisulfite, 1.1 mg thiamin HCl, 5.5 mg riboflavin, 16.5 mg D-calcium pantothenate, 66 ng niacin, 750 mg choline chloride, .015 mg vitamin B_{12}, 1.1 mg folic acid, .22 mg biotin, 2.2 mg pyridoxine HCl, 125 mg ethoxyquin, .2 mg selenium from sodium selenite, and 55 mg penicillin from procaine penicillin.

TABLE 2.2. Composition of the basal diet[1]

Ingredient	Amount
	(g/kg)
Ground yellow corn	404.4
Stabilized fat	30.0
Dehulled soybean meal	520.0
Ground limestone	35.0
Iodized salt	5.0
Trace mineral mix[2]	.7
Vitamins and feed additives[3]	2.9
DL-Methionine	2.0
Total	1000.0

[1]Contained by calculation using values from National Research Council (1984): 1.48% calcium and .44% total phosphorus.

[2]Supplied per kilogram of diet: 150 mg manganese, 100 mg zinc, 70 mg iron, 10 mg copper, and 1 mg iodine from manganese oxide, zinc oxide, ferrous sulfate heptahydrate, anhydrous cupric sulfate, and potassium iodate, respectively.

[3]Supplied per kilogram of diet: 6,600 IU vitamin A, 3,300 ICU vitamin D_3, 11 IU vitamin E, 7 mg menadione sodium bisulfite, 1.1 mg thiamin HCl, 5.5 mg riboflavin, 16.5 mg D-calcium pantothenate, 66 ng niacin, 750 mg choline chloride, .015 mg vitamin B_{12}, 1.1 mg folic acid, .22 mg biotin, 2.2 mg pyridoxine HCl, 125 mg ethoxyquin, .2 mg selenium from sodium selenite, and 55 mg penicillin from procaine penicillin.

TABLE 2.3. Mortality during the 3-week experiment

Added phosphorus	Dynafos		Multifos	
	Male	Female	Male	Female
(%)		Experiment 1		
.09	2/8[1]	1/10	0/8	2/10
.18	0/8	1/10	1/8	1/10
.27	0/8	0/10	1/8	0/10
.45	2/8	1/10	0/8	0/10
		Experiment 2		
.09	1/9	0/9	1/9	2/9
.18	1/9	0/9	0/9	0/9
.27	0/9	0/9	0/9	0/9
.45	0/9	0/9	0/9	0/9

[1]Number of deaths/total number of birds.

TABLE 2.4. Average body weight gain from 1 to 3 weeks of age

Added phosphorus	Dynafos		Multifos	
	Male	Female	Male	Female
(%)	(g)			
	Experiment 1			
.09	309.5	265.0	292.4	290.5
.18	362.5	309.8	338.0	300.4
.27	408.9	343.1	380.6	343.6
.45	469.2	416.0	437.1	398.5
	Experiment 2			
.09	318.7	285.9	282.9	254.2
.18	346.6	330.1	354.0	305.4
.27	381.4	330.1	397.4	334.4
.45	411.6	370.4	457.7	361.0

TABLE 2.5. Average body weight gain from 1 to 4 weeks of age

Added phosphorus	Dynafos		Multifos	
	Male	Female	Male	Female
(%)	(g)			
	Experiment 1			
.09	494.5	457.9	502.4	479.9
.18	589.7	529.9	557.1	503.4
.27	672.7	576.3	604.9	540.9
.45	814.7	655.2	711.6	632.5
	Experiment 2			
.09	508.0	447.4	437.5	421.0
.18	616.0	566.3	610.9	526.9
.27	695.4	595.0	712.2	598.7
.45	736.6	667.9	762.6	616.3

TABLE 2.6. Ash content of dry toes from poults at 4 weeks of age

Added phosphorus	Dynafos		Multifos	
	Male	Female	Male	Female
(%)	(%)			
	Experiment 1			
	Middle toe			
.09	8.59	8.54	8.31	8.58
.18	9.95	10.15	8.86	10.08
.27	11.32	11.26	10.88	11.22
.45	12.75	12.54	12.86	12.40
	Inside toe			
.09	9.14	9.19	8.78	8.95
.18	10.64	11.06	10.83	10.94
.27	11.88	12.13	11.71	11.80
.45	14.15	13.32	13.74	13.19
	Experiment 2			
	Middle toe			
.09	7.35	7.92	7.48	7.01
.18	9.74	9.95	9.26	8.69
.27	11.37	11.36	10.58	10.90
.45	12.70	12.55	12.30	12.45
	Inside toe			
.09	8.45	8.60	8.83	8.01
.18	10.78	10.76	10.13	9.52
.27	11.94	11.89	11.58	11.44
.45	13.33	12.94	13.12	12.77

TABLE 2.7. Relative biological value of phosphorus from Multifos to phosphorus from Dynafos using 4-week toe ash data

Experiment	Toe	Sex	Error df	Relative biological value	95% Confidence limits
1	Middle	Male	4	88.4	...[1]
	Middle	Female	4	97.7	94-102
	Middle	Both	12	91.4	...
	Inside	Male	4	92.5	86-101
	Inside	Female	4	91.9	84-101
	Inside	Both	12	92.1	68-130
2	Middle	Male	4	87.1	75-102
	Middle	Female	4	78.9	59-108
	Middle	Both	12	83.2	70-100
	Inside	Male	4	91.2	73-116
	Inside	Female	4	78.5	63- 98
	Inside	Both	12	85.0	65-113

[1]Confidence limit calculation was not possible ($-\infty$ to $+\infty$).

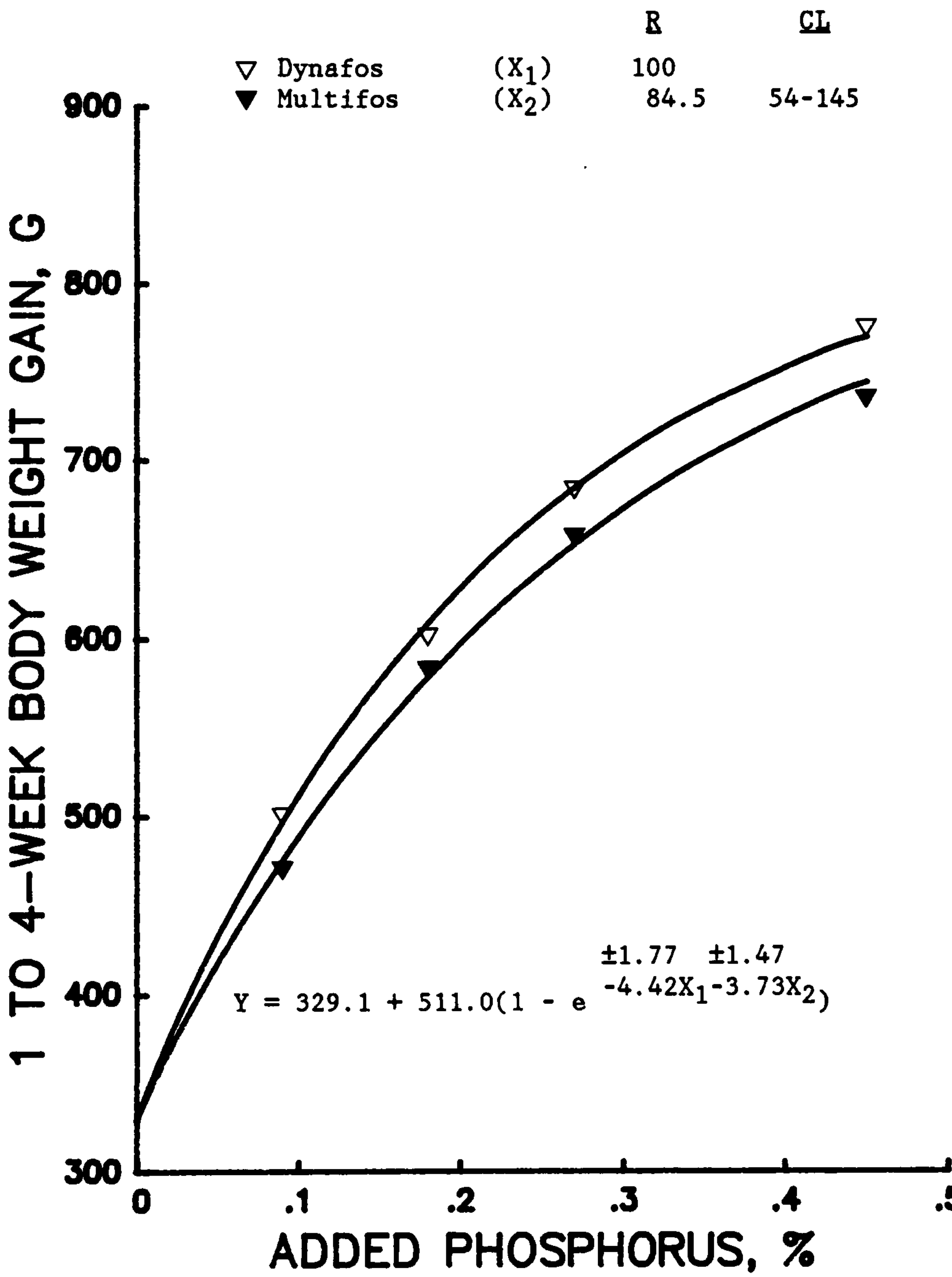

FIGURE 2.1. Plot of body weight gain of male turkeys from 1 to 4 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL).

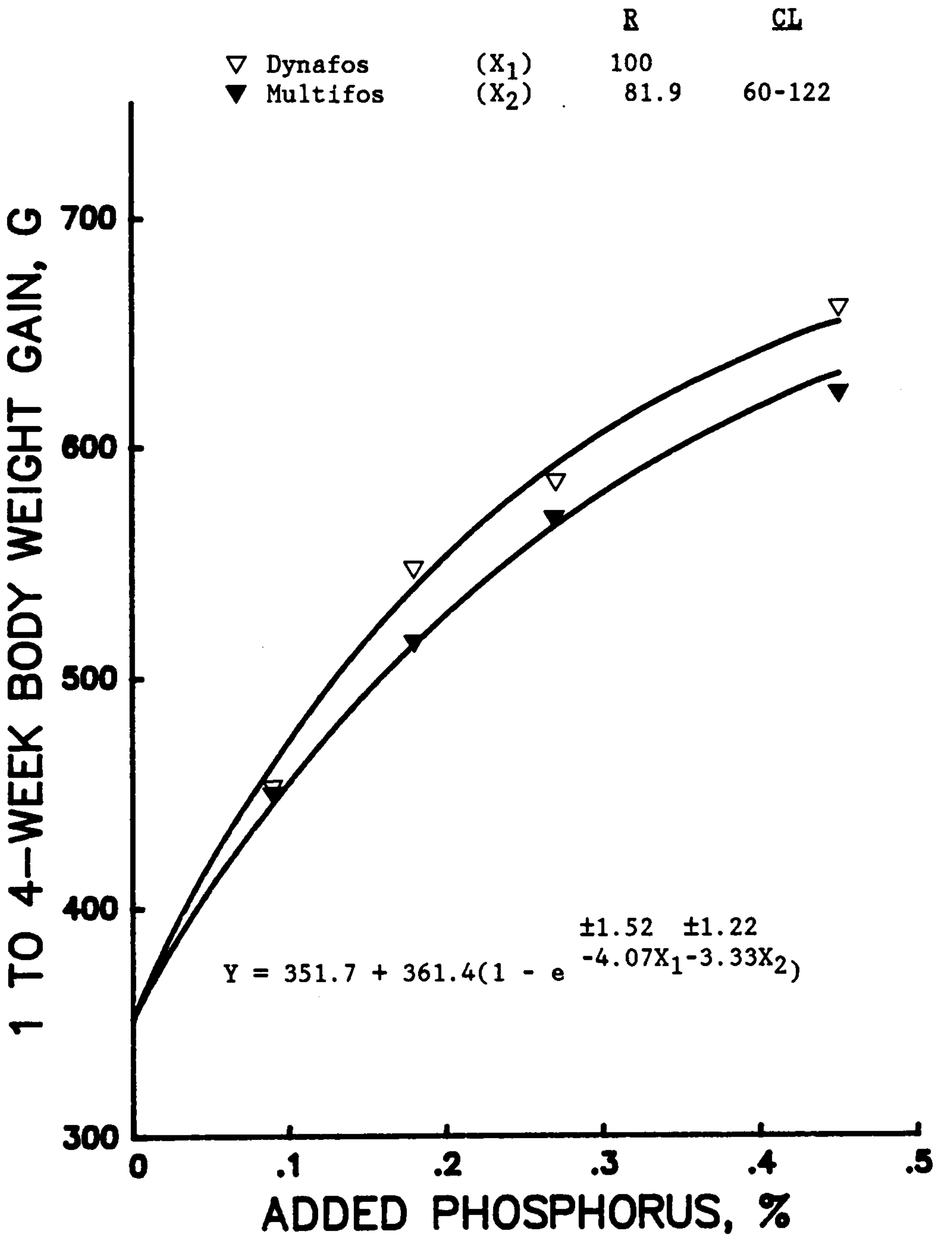

FIGURE 2.2. Plot of body weight gain of female turkeys from 1 to 4 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL).

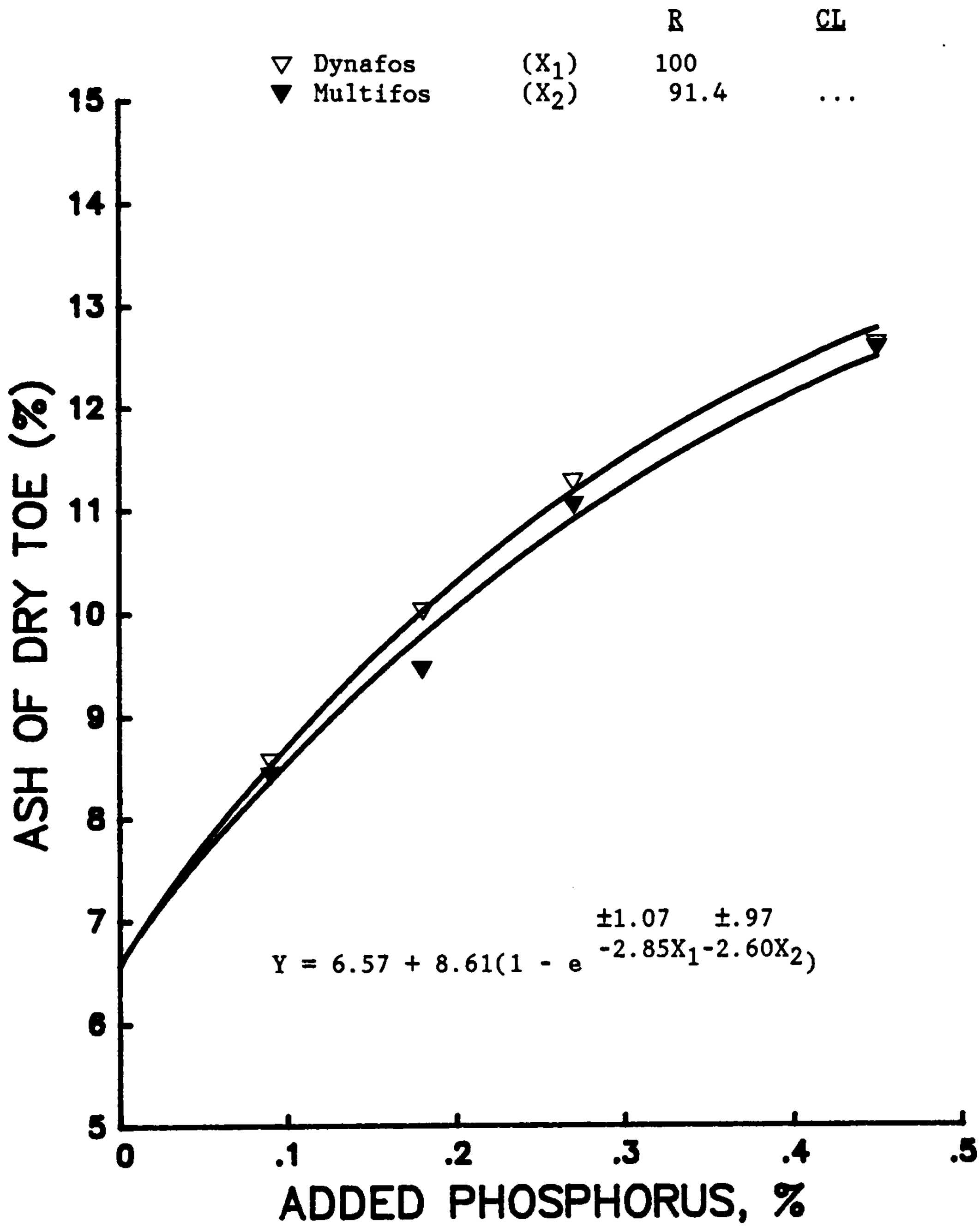

FIGURE 2.3. Plot of ash content of middle toes of turkeys at 4 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL) in Experiment 1.

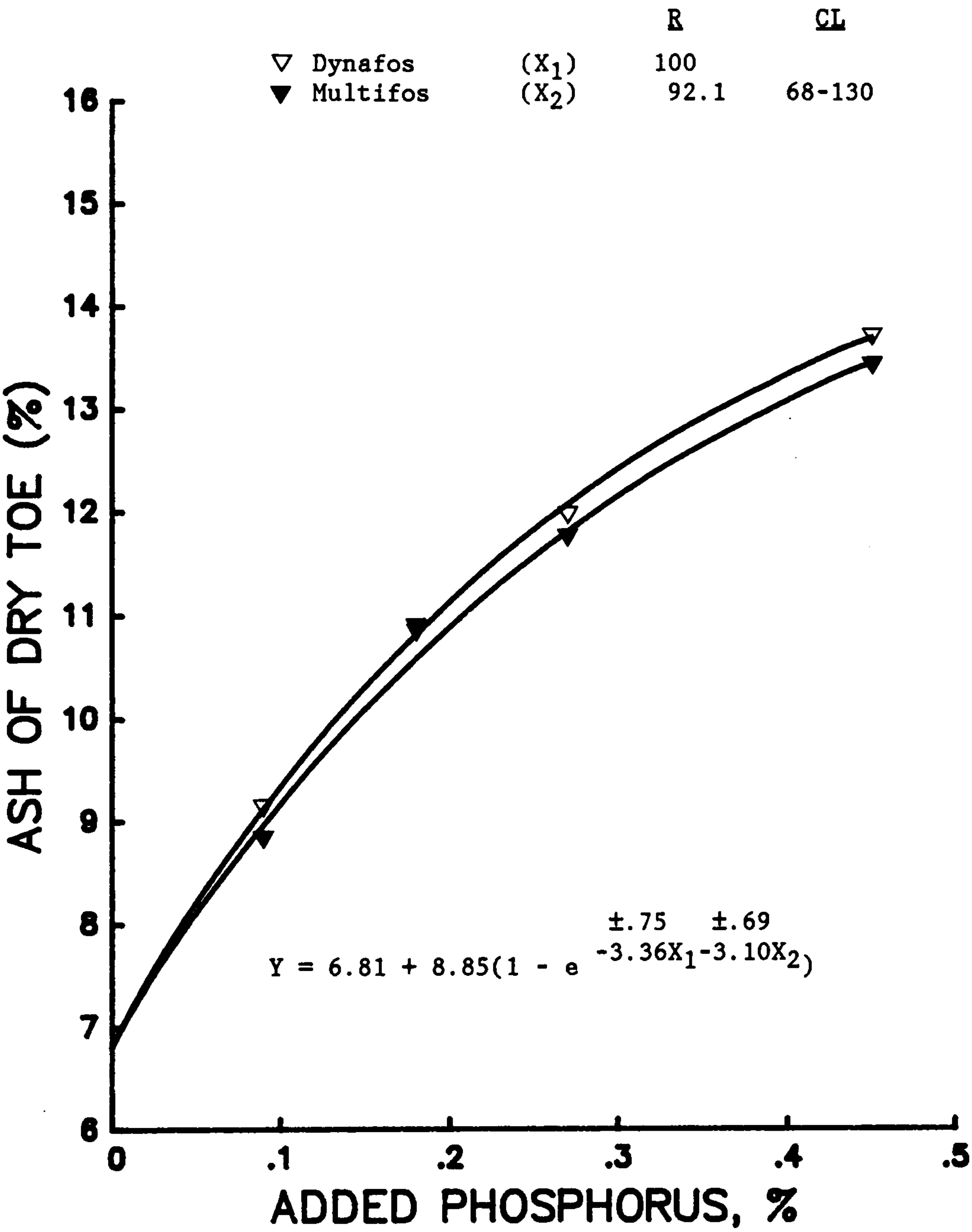

FIGURE 2.4. Plot of ash content of inside toes of turkeys at 4 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL) in Experiment 1.

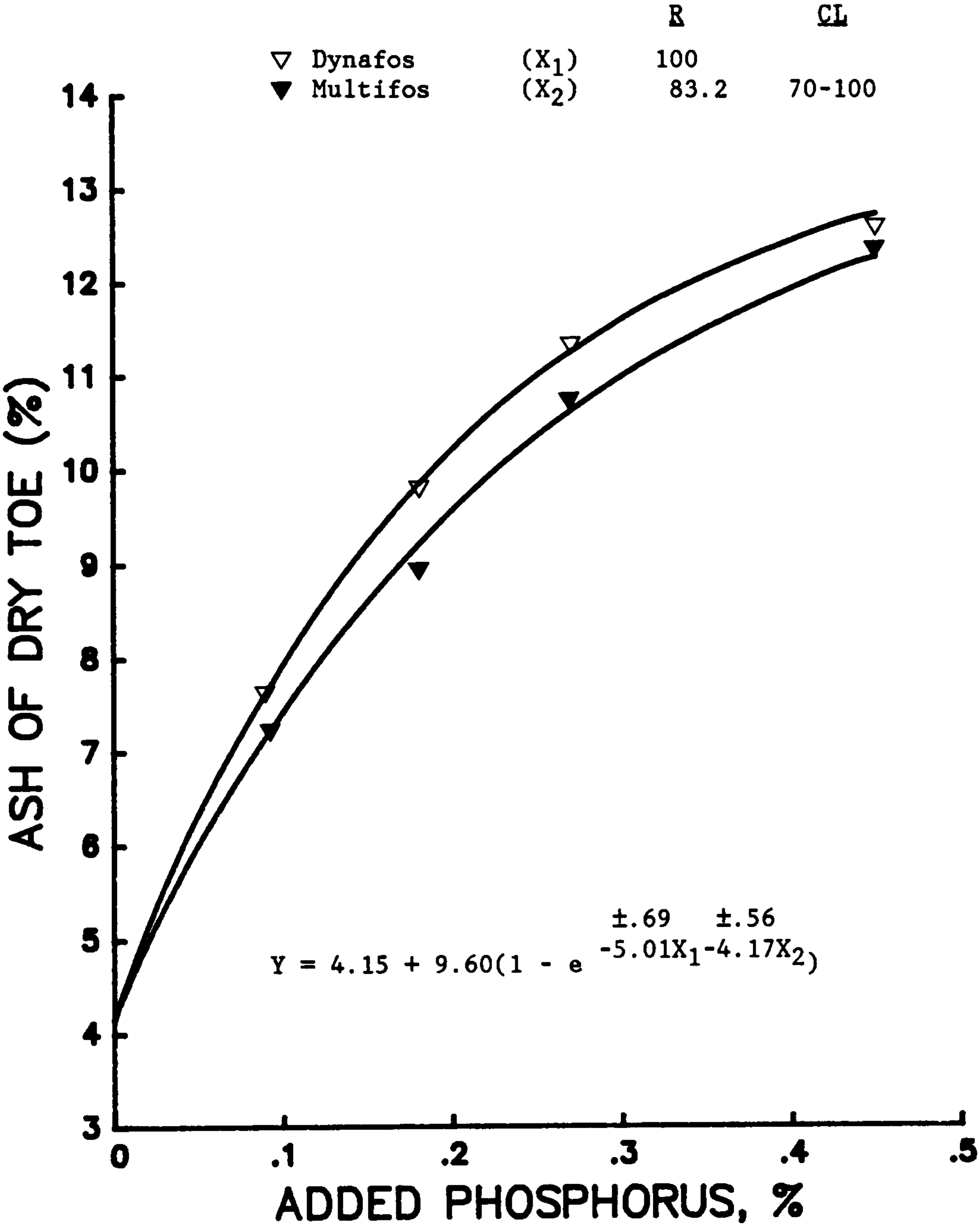

FIGURE 2.5. Plot of ash content of middle toes of turkeys at 4 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL) in Experiment 2.

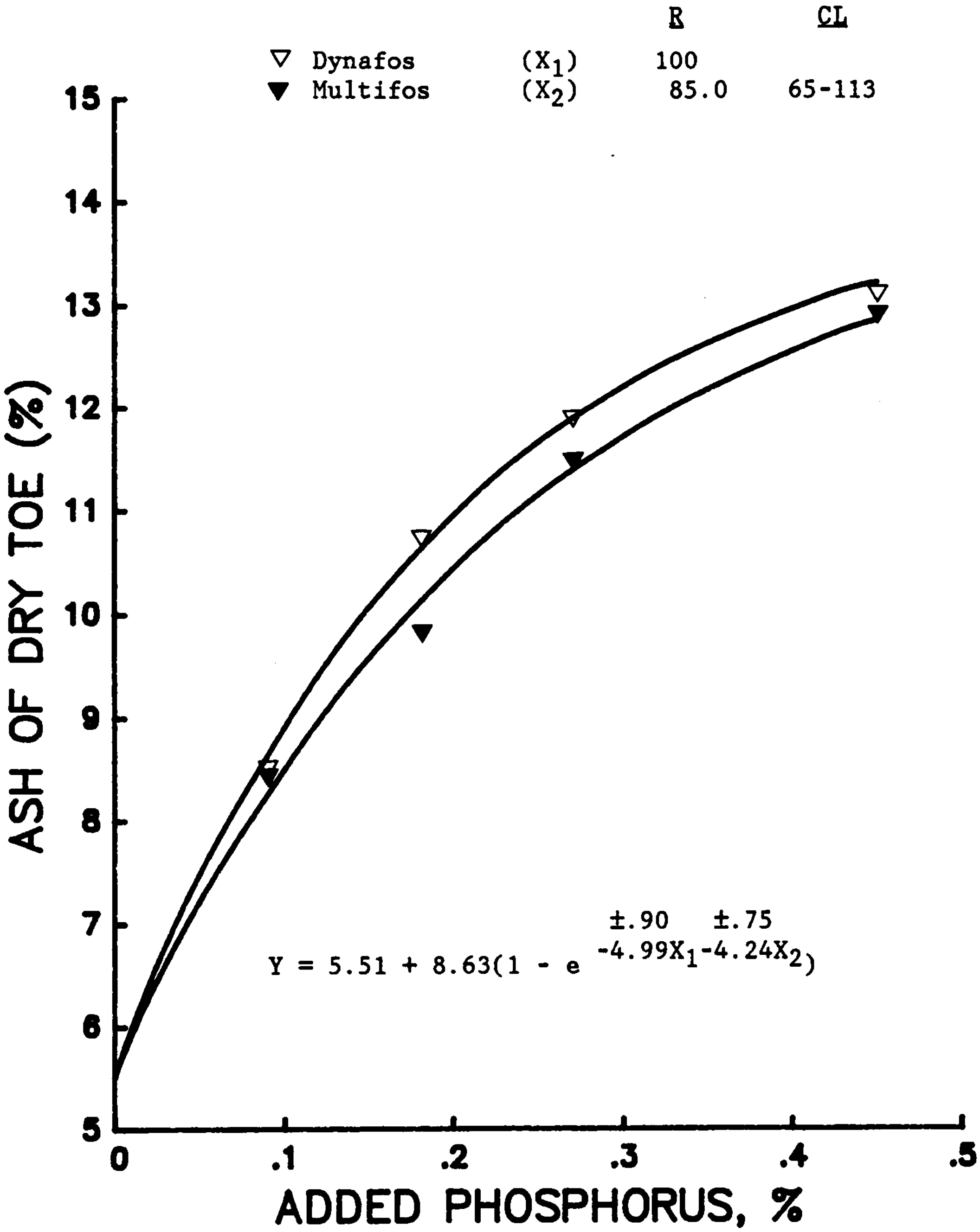

FIGURE 2.6. Plot of ash content of inside toes of turkeys at 4 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL) in Experiment 2.

SERIES III. EVALUATION OF GROWTH RESPONSE TO LEVELS OF ADDED PHOSPHORUS AND RELATIVE BIOAVAILABILITY DETERMINATION OF VARIOUS DEFLUORINATED PHOSPHATE PRODUCTS

Results from Series I demonstrated significant differences between bioavailability of phosphorus from various sources, with defluorinated phosphates from two different groups (seven products) being 70 and 76% as available as the standard dicalcium phosphate dihydrate. An asymptotic regression equation was found to be the best fit for the analyses when both body weight and toe ash data were used.

Three experiments were conducted to determine relative bioavailability of various phosphate products. More levels of added phosphorus were used in these experiments to obtain a more precise growth response to the added phosphorus.

EXPERIMENT 1

This experiment was conducted to determine relative bioavailability of a pre-commercial defluorinated phosphate as compared with a $CaHPO_4 \cdot 2H_2O$ standard, and to obtain a more precise growth curve in response to levels of phosphorus added.

MATERIALS AND METHODS

The pre-commercial defluorinated phosphate tested contained 32.72% calcium, 18.10% phosphorus, 4% sodium, and .13% fluorine. The standard phosphate used in this experiment was a dicalcium phosphate dihydrate from J. T. Baker Chemical Company (USP grade)[2] and was found to contain 23.65% calcium and 18.29% phosphorus.

The composition of the basal diet used in this experiment was equivalent to that used in Series I (Table 1.1). Twelve levels of inorganic phosphorus were used to define the growth curve. The phosphates were added to the basal diet to supply .09, .11, .13, .15, .18, .21, .24, .27, .36, .45, .54, and .72% phosphorus. Dietary calcium and sodium contents were held constant at 1.48 and .22%, respectively.

Two replicates of 24 pens with 10 male Large White poults per pen were used in this experiment. The poults in each replicate were randomized into one Petersime battery at day one. Birds were group-weighed at the beginning of the experiment and at weekly intervals to 3 weeks of age when they were sacrificed and right tibiae and left and right middle toes were collected for analyses (A.O.A.C., 1984). Tibiae were cleaned of adhering tissue, dried at 105 C for 24 hours, cooled to room temperature in a desiccator, and weighed, and the right tibiae were ashed at 550 C for 12 hours. The tibia ash content was expressed on a

---

[2] A J. T. Baker Chemical Company product, USP grade, was used in Experiment 1 because insufficient amount of the dihydrate dicalcium phosphate from Series I, a Fisher Scientific Company product, purified grade, remained from an earlier purchase for use as a standard in that experiment and the product has since been discontinued by the manufacturer.

dry unextracted basis. Middle toes collected from birds in the same pen were cleaned, dried at 105 C for 18 hours, cooled to room temperature, weighed, and ashed for 4 hours at 600 C in group composite with toes from left and right feet ashed separately.

Body weight gain, toe ash, and tibia ash were subjected to analyses of variance and nonlinear regression analyses (Noll, 1984). A sigmoidal regression analysis (Robbins et al., 1979) was also applied to all data and a significant reduction in mean squares of the residual term was used to determine the appropriateness of the regression analysis.

RESULTS

Mortality of poults during the 3-week experiment is presented in Table 3.1. From the mortality data alone, it was obvious that the defluorinated phosphate was much less available to the poults than the standard dicalcium phosphate dihydrate with mortality in birds fed the defluorinated phosphate totalling 48 and 46 birds in the two replicates and in birds fed the standard phosphate 12 and 12 birds in the two replicates.

The average body weight gain from 0 to 2 weeks of age is presented in Table 3.2. The body weight gain from feeding the defluorinated phosphate was consistently lower than that from feeding the standard phosphate. Data from both replicates were combined for analyses. A reduction in residual sum of squares was significant from linear to asymptotic regression models but was not significant from asymptotic to sigmoidal regression models (Table 3.3). The asymptotic regression equation provided a significantly better fit to the 2-week body weight gain data than the linear regression equation but no further gain in precision was observed with the sigmoidal regression equation. Plot of 2-week body weight gain on levels of added phosphorus is presented in Figure 3.1.

The average body weight gain from 0 to 3 weeks of age (Table 3.4) was also consistently lower in birds fed defluorinated phosphate than in birds fed the standard dicalcium phosphate dihydrate. Analyses of combined data resulted in a sigmoidal regression equation giving a significantly better fit than an asymptotic regression equation (Table

3.5). Plot of 3-week body weight gain on levels of added phosphorus is presented in Figure 3.2.

The average ash contents of dry toes from poults at 3 weeks of age are presented in Table 3.6. Again, the toe ash contents of poults fed defluorinated phosphate were consistently lower than those of poults fed the standard phosphate. A sigmoidal regression equation was also found to provide the best fit equation of the three regression equations tested (Table 3.7).

The average ash contents of dry unextracted tibiae of poults at 3 weeks of age are presented in Table 3.8 and the analysis of variance is presented in Table 3.9. A sigmoidal regression equation was again found to provide the best fit. Plots of toe ash and tibia ash on levels of added phosphorus are presented in Figures 3.3 and 3.4, respectively.

DISCUSSION

Biological values of phosphorus in the tested defluorinated phosphate were significantly lower than that in the standard dicalcium phosphate dihydrate in all cases. The 2-week body weight gain data proved to be sufficient in detecting the difference in bioavailability of the phosphorus sources and appeared to have a slightly narrower confidence limit than the 3-week body weight gain data when the asymptotic regression analysis of 2-week data was compared with the sigmoidal analysis of the 3-week data. The relative biological value of the defluorinated phosphate from 2-week body weight gain was 50% while that from 3-week body weight gain was 52%. The difference was not statistically significant.

Tibia bones were normally collected with both cartilage caps and extracted for ash determination. Evans and St. John (1944) determined the ash contents of the tibia shaft and the distal and proximal cartilage of 3-week-old chicks and found the tibia shaft to approach a saturation point at a lower dietary vitamin D level than either cartilage. Toe ash was found to increase in the same manner as the cartilages within the range of vitamin D supplementations in the study. They did not dispute the suggestion of Johnson (1942) and Fritz and Halloran (1943) that the shaft could replace the tibia in the ash determination for vitamin D bioassay and noted that difficulty in separating the shaft from the cartilage may be responsible for the results obtained in their study. They concluded that the bioavailability of vitamin D obtained from the ash content in the shaft, the distal cartilage, the extracted toes, and the unextracted toes compared favorably to the value obtained from the tibia ash. The use of unextracted tibia was suggested but was not further investigated.

In the present study, the cleaning of the tibia was done per suggestion of A.O.A.C. (1984). The cartilage caps were easily separated from the shaft after the tibia was placed in boiling water for less than 2 minutes. The cartilage caps were damaged during the bone collections and their inclusion may have unnecessarily inflated the experimental error.

The 3-week toe ash and the 3-week tibia ash data yielded almost identical biological values as well as confidence limits for the tested defluorinated phosphate. Values of using the toe ash determination as compared with the tibia ash for bioassay were confirmed when a slope ratio bioassay was involved with toe ash being slightly more sensitive than tibia ash in most experiments (Evans and St. John, 1944; Fritz and Roberts, 1968; Yoshida and Hoshii, 1977; 1978). Even though toe ash was not found to be more sensitive in the current experiment, it was found to be equal to tibia ash in the phosphorus bioassay when nonlinear regression was used in the relative bioavailability analyses. Both toe ash and tibia ash data yielded better precision than the body weight gain data for biological value determination of phosphorus in this experiment. Nelson (1967) found that the bone ash was one of the most sensitive practical criteria for evaluation of phosphorus utilization, and the body weight gain was not an accurate measure and had led to misleading conclusions. Sullivan (1966) gave priority to body weight gain data in the triple response method for phosphorus evaluation. Body weight gain accounted for 55% of the biological value calculation while bone ash was allowed 40%. Feed efficiency, which is also a growth parameter, accounted for the other 5%.

An asymptotic regression analysis was introduced by Noll et al. (1984) for use in methionine bioassay. A growth process over time was an exponential response with a saturation effect as an animal nears maturity making the composite response a sigmoidal equation. A growth response was found to be an asymptotic regression to feed consumption (Park, 1970). Park (1971) reported an asymptotic growth response to dietary methionine in chicks from 1 to 3 weeks of age. Robbins et al. (1979) agreed with the asymptotic response to feeding but added that the sigmoidal response may be possible if all doses of nutrient were possible in the growth trials. The current experiment showed sigmoidal responses to added phosphorus in data at 3 weeks of age. Two-week body weight gain did not exhibit a sigmoidal response to added phosphorus but relative biological value and its confidence limit calculated from the 2-week gain data were equal to one calculated from the sigmoidal response of the 3-week body weight gain data. By using more levels of added phosphorus, the bioassay may be shortened to a period of 2 weeks without compromising the precision of the assay. Although the ash contents at 2 weeks of age with more levels of added phosphorus were not tested in this experiment, they should provide an equally precise bioassay of phosphorus as the 3-week ash data.

TABLE 3.1. Mortality during the 3-week experiment

Added phosphorus	DFP[1]		Standard	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2
(%)				
.09	9[2]	10	4	4
.11	9	8	1	5
.13	7	8	2	2
.15	8	7	1	0
.18	4	6	0	0
.21	6	5	0	0
.24	3	1	0	0
.27	2	1	2	0
.36	0	0	0	0
.45	0	0	1	1
.54	0	0	0	0
.72	0	0	1	0

[1]DFP = Defluorinated phosphate.

[2]Number of deaths from ten poults per pen.

TABLE 3.2. Average body weight gain from 0 to 2 weeks of age

Added phosphorus	DFP[1]		Standard	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2
(%)	(g)			
.09	89.4	84.0	135.6	150.8
.11	94.3	106.4	164.0	176.0
.13	111.3	121.8	179.4	183.5
.15	111.8	119.9	177.8	212.9
.18	137.5	140.2	163.1	210.1
.21	149.9	145.3	204.7	209.2
.24	158.1	166.2	217.9	207.3
.27	170.3	164.8	206.9	219.8
.36	218.4	197.9	242.1	217.0
.45	230.4	225.5	248.1	257.4
.54	267.1	231.1	242.6	250.9
.72	250.5	226.7	214.2	230.2

[1]DFP = Defluorinated phosphate.

TABLE 3.3. Analysis of variance of 0 to 2-week body weight gains from linear, asymptotic, and sigmoidal regressions

Source	Degrees of freedom	Sum of squares	Mean squares
Total about origin	48	1,743,942	
Mean	1	1,628,144	
Total about mean	47	115,798	
Linear regression	2	69,821	
Residual	45	45,978	1,022
Asymptotic regression	4	1,735,230	
Residual	44	8,712	198
Sigmoidal regression	5	1,735,356	
Residual	43	8,586	200
Reduction due to			
Asymptotic	1	37,266	37,266***
Sigmoidal	1	126	126

***P<.001.

TABLE 3.4. Average body weight gain from 0 to 3 weeks of age

Added phosphorus	DFP[1]		Standard	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2
(%)	(g)			
.09	173.2	...[2]	227.2	224.2
.11	180.7	141.4	245.2	252.7
.13	210.3	179.1	334.8	339.0
.15	186.0	186.8	288.6	368.7
.18	223.9	144.2	345.8	364.6
.21	239.1	216.3	391.2	382.2
.24	322.1	273.2	426.2	386.1
.27	315.3	298.5	449.9	419.9
.36	408.5	387.8	446.0	432.7
.45	446.0	439.2	473.2	493.2
.54	498.8	427.4	471.9	483.7
.72	456.6	435.7	422.8	454.2

[1]DFP = Defluorinated phosphate.

[2]All poults died.

TABLE 3.5. Analysis of variance of 0 to 3-week body weight gains from linear, asymptotic, and sigmoidal regressions

Source	Degrees of freedom	Sum of squares	Mean squares
Total about origin	47	5,937,285	
Mean	1	5,388,480	
Total about mean	46	548,805	
Linear regression	2	344,774	
Residual	44	204,031	4,637
Asymptotic regression	4	5,894,882	
Residual	43	42,403	986
Sigmoidal regression	5	5,904,579	
Residual	42	32,706	779
Reduction due to			
Asymptotic	1	161,628	161,628***
Sigmoidal	1	9,697	9,697**

**P<.01.

***P<.001.

TABLE 3.6. Average ash content of dry toes from poults at 3 weeks of age

Added phosphorus	DFP[1]		Standard	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2
(%)	(%)			
.09	4.11	...[1]	5.54	5.93
.11	4.98	3.88	6.39	7.01
.13	4.54	4.68	6.90	7.86
.15	4.43	5.92	7.49	8.25
.18	6.05	5.56	9.61	9.39
.21	7.54	6.44	10.75	10.25
.24	7.26	7.35	10.89	10.94
.27	8.12	7.73	11.85	10.89
.36	10.57	10.46	11.57	12.06
.45	11.58	10.89	12.00	11.56
.54	11.64	10.98	11.77	11.89
.72	11.13	11.36	12.00	12.43

[1]DFP = Defluorinated phosphate.

[2]All poults died.

TABLE 3.7. Analysis of variance of 3-week toe ash from linear, asymptotic, and sigmoidal regressions

Source	Degrees of freedom	Sum of squares	Mean squares
Total about origin	47	3,995.9	
Mean	1	3,656.6	
Total about mean	46	339.3	
Linear regression	2	227.8	
Residual	44	111.5	2.53
Asymptotic regression	4	3,942.7	
Residual	43	13.2	.31
Sigmoidal regression	5	3,948.3	
Residual	42	7.5	.18
Reduction due to			
Asymptotic	1	98.3	98.3***
Sigmoidal	1	5.7	5.7***

***P<.001.

TABLE 3.8. Average ash content of dry unextracted tibiae from poults at 3 weeks of age

Added phosphorus	DFP[1]		Standard	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2
(%)	(%)			
.09	32.21	...[2]	34.60	34.78
.11	28.97	30.80	36.17	38.98
.13	30.47	33.15	37.12	36.30
.15	32.58	34.42	39.83	38.81
.18	34.63	34.58	43.02	43.03
.21	35.27	35.55	44.31	44.99
.24	37.32	38.02	45.76	45.88
.27	40.10	39.07	48.00	47.93
.36	45.19	45.42	49.92	50.40
.45	47.20	47.22	49.83	49.92
.54	48.59	48.73	49.17	49.81
.72	48.41	47.73	49.32	49.46

[1]DFP = Defluorinated phosphate.

[2]All poults died.

TABLE 3.9. Analysis of variance of 3-week tibia ash from linear, asymptotic, and sigmoidal regressions

Source	Degrees of freedom	Sum of squares	Mean squares
Total about origin	47	83,191.9	
Mean	1	81,150.9	
Total about mean	46	2,041.0	
Linear regression	2	1,411.2	
Residual	44	629.8	14.31
Asymptotic regression	4	83,111.4	
Residual	43	80.5	1.87
Sigmoidal regression	5	83,147.5	
Residual	42	44.4	1.06
Reduction due to			
Asymptotic	1	549.3	549.3***
Sigmoidal	1	36.1	36.1***

***P<.001.

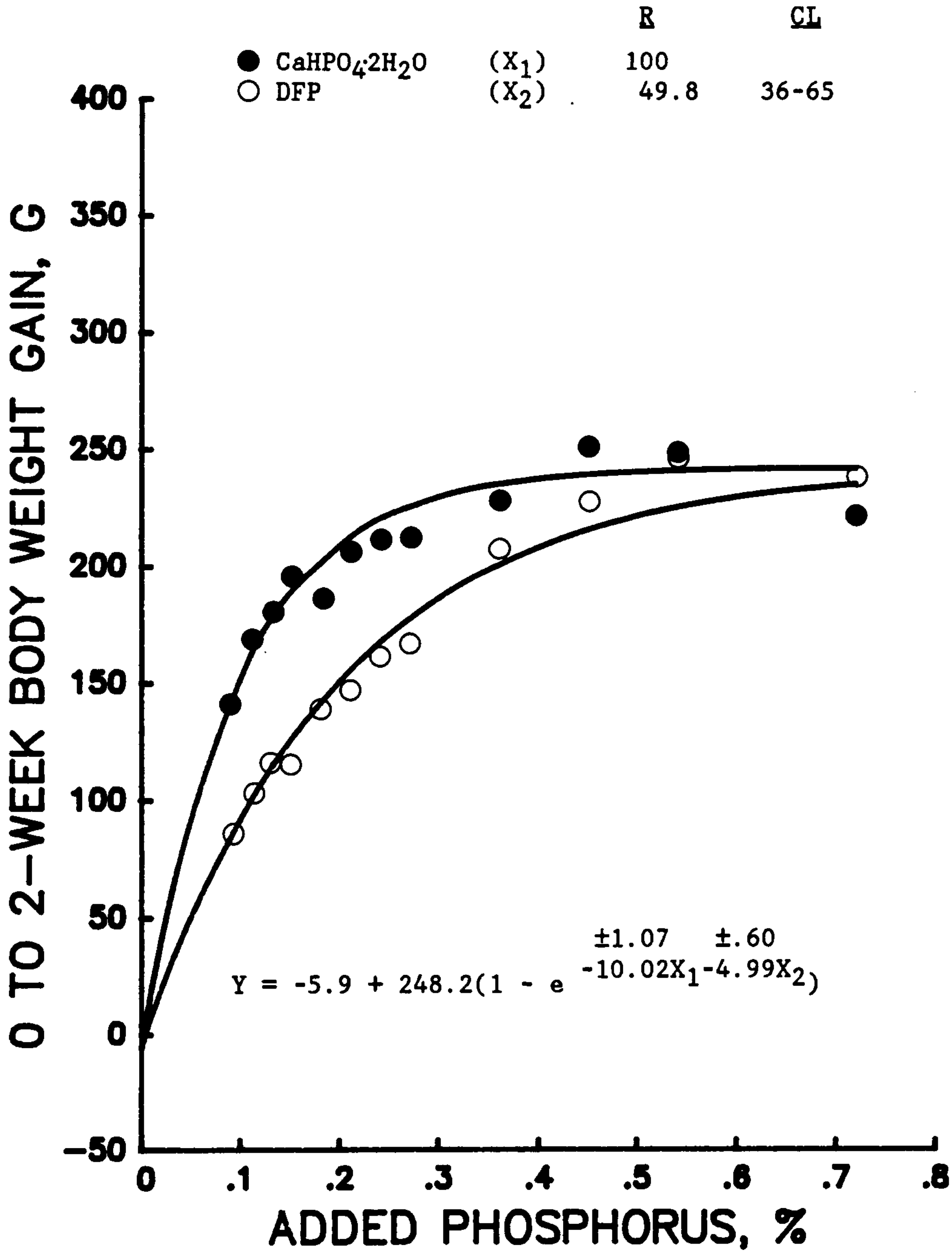

FIGURE 3.1. Plot of body weight gain of turkeys from 0 to 2 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL). DFP=Defluorinated phosphate.

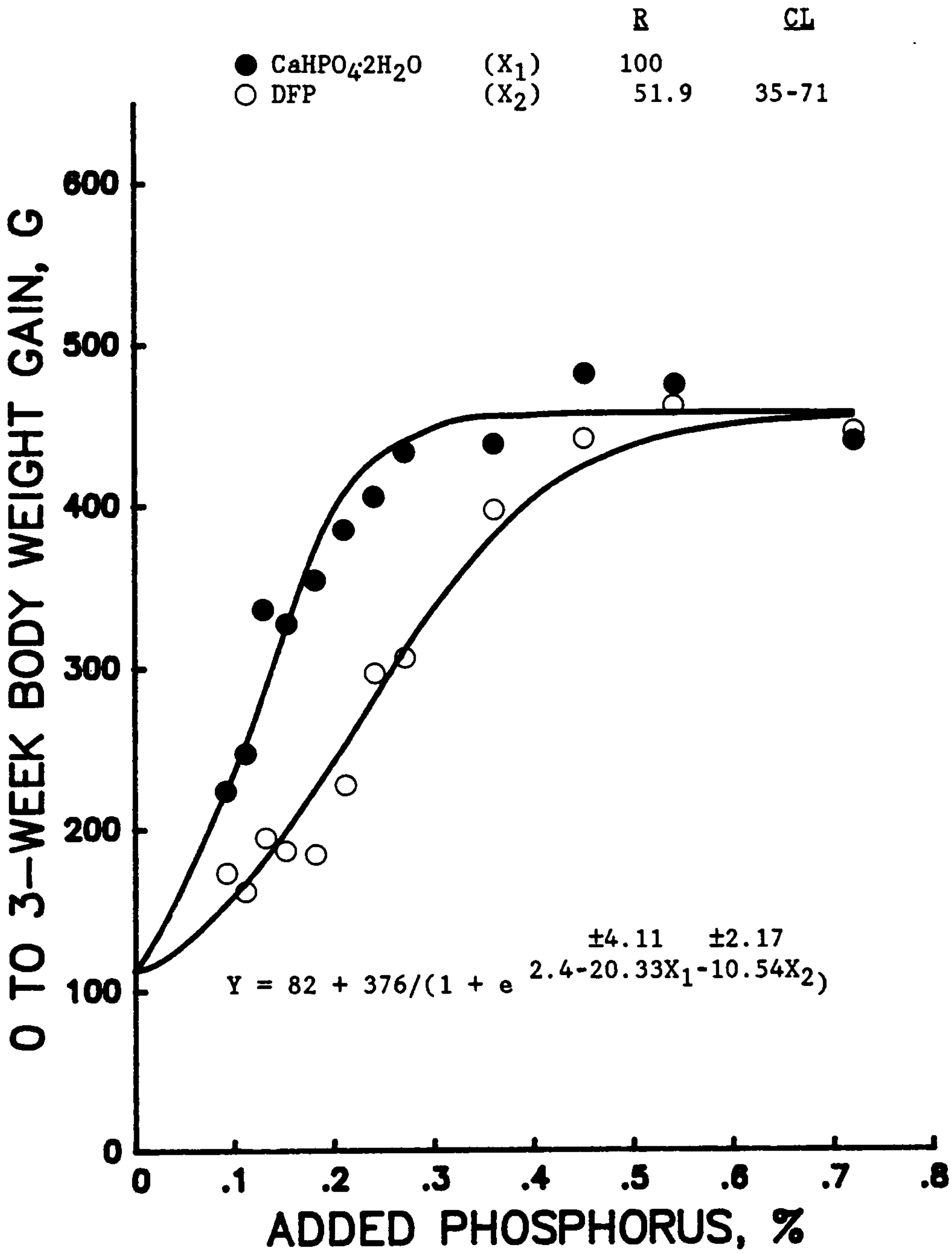

FIGURE 3.2. Plot of body weight gain of turkeys from 0 to 3 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL). DFP=Defluorinated phosphate.

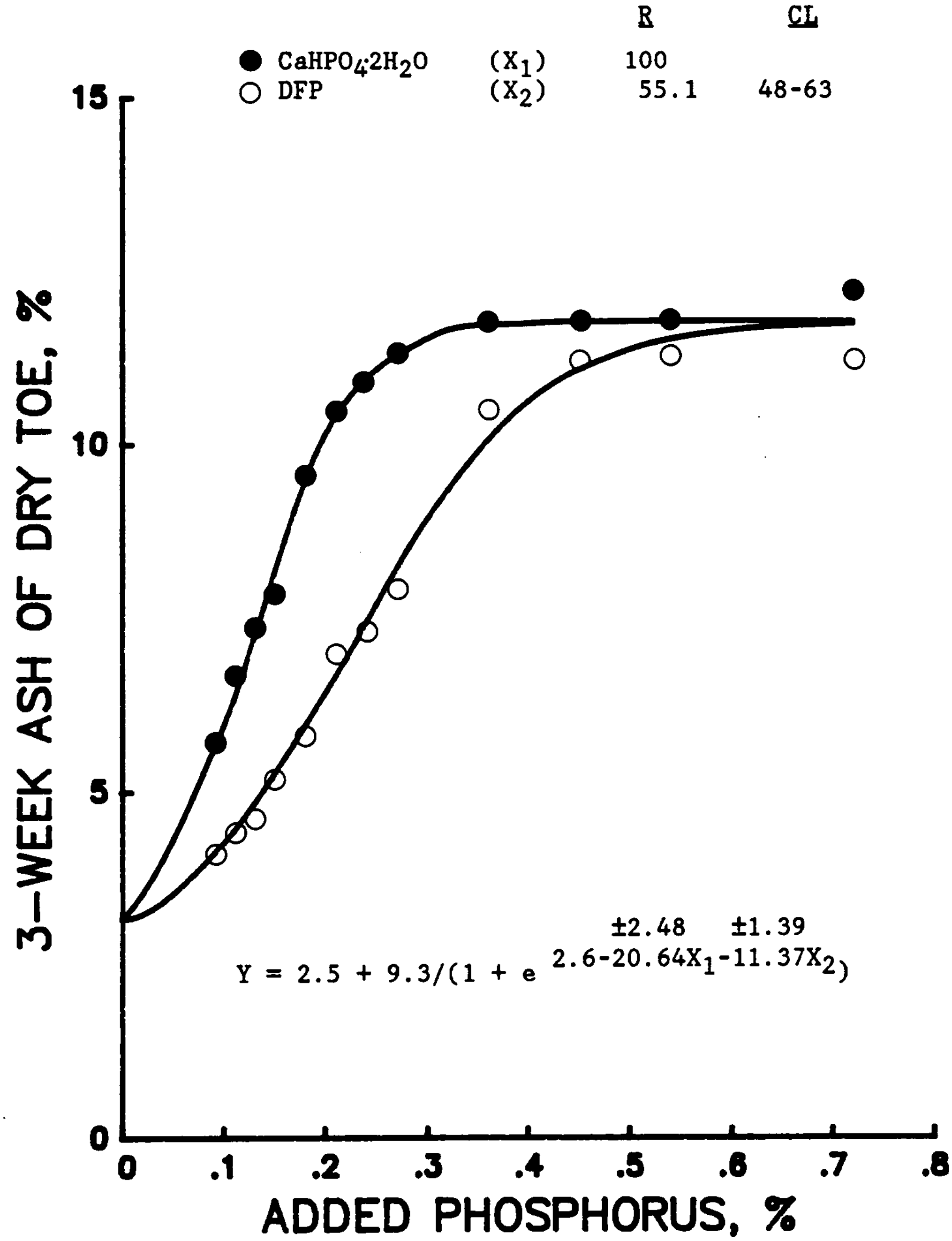

FIGURE 3.3. Plot of ash content of dry toes of turkeys at 3 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL). DFP=Defluorinated phosphate.

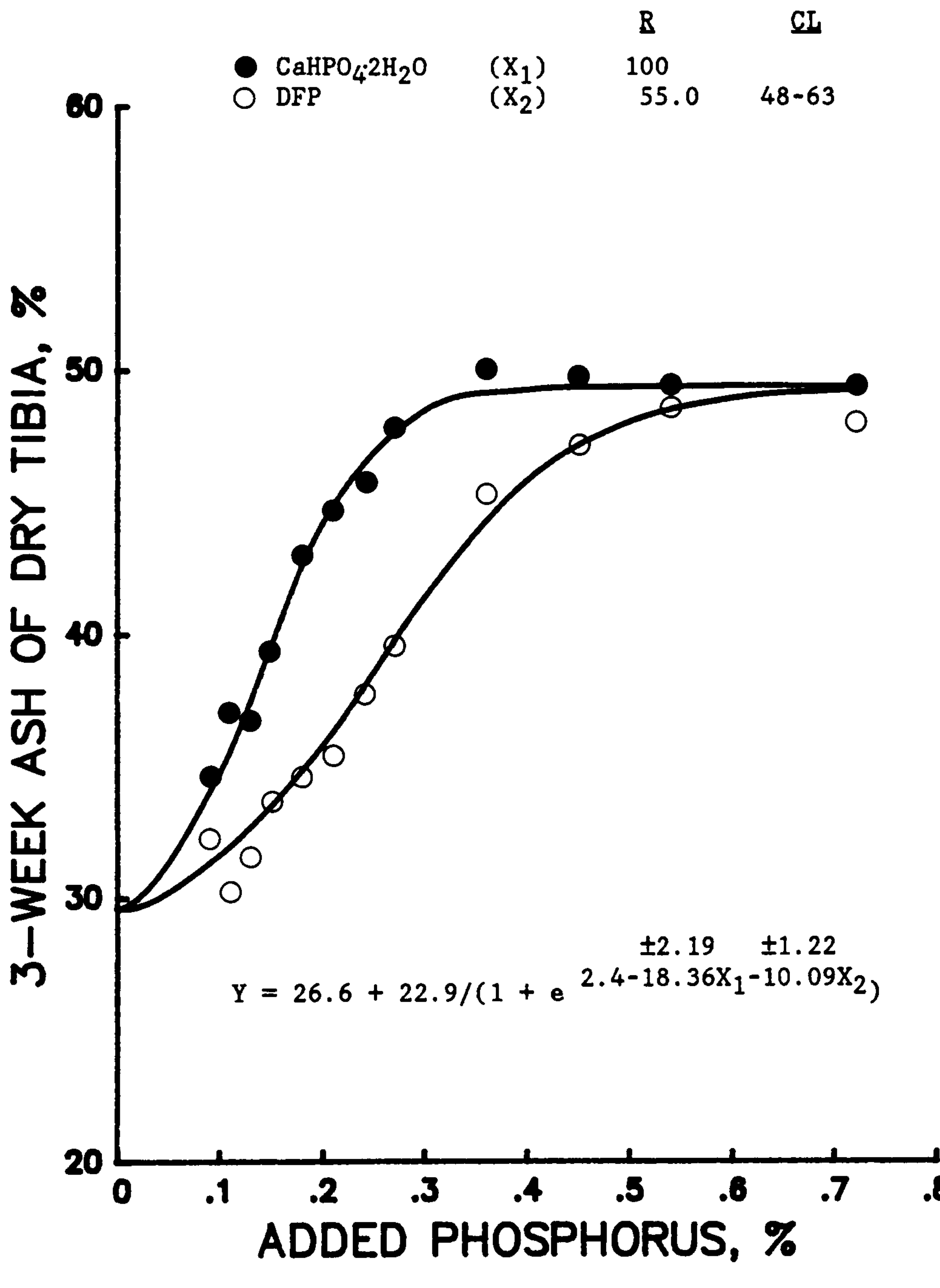

FIGURE 3.4. Plot of ash content of dry unextracted tibiae of turkeys at 3 weeks of age on level of added phosphorus and relative biological value (R) with 95% confidence limit (CL). DFP=Defluorinated phosphate.

EXPERIMENT 2

In Series I, phosphorus in four samples of "experimental" defluorinated phosphates obtained from pre-commercial production was evaluated and found to be 75.7% as available as that in dihydrate dicalcium phosphate. The phosphorus in these samples averaged 18% less available ($P<.001$) than that in three samples of mono/dicalcium phosphate and slightly (7%) less available ($P<.05$) than that in three samples of dicalcium phosphate, but slightly (8%) more available ($P<.05$) than that in three samples of commercial defluorinated phosphate.

In Experiment 1 of Series III, the phosphorus in a sample of pre-commercial defluorinated phosphate was evaluated. In comparison to dihydrate dicalcium phosphate, the defluorinated phosphate was found to be only 55.1% available using toe ash measurements at 3 weeks of age (lower and upper confidence limits were 52 and 58%). This value is considerably lower than that of 75.7% obtained from the phosphorus in the experimental defluorinated phosphate in Series I. The cause of the difference in bioavailability of the phosphorus in these defluorinated phosphates produced in the same production plant is not known and is of great importance. No other phosphates were tested in Experiment 1. The source of the dicalcium phosphate dihydrate used in Experiment 1 was from J. T. Baker Chemical Company whereas that used in Series I was from Fisher Scientific Company. The difference between the bioavailability of 75.7 and 55.1% may be due to a difference in the standards, to a difference in the biological value of the experimental _vs_. the pre-commercial products, or to some source of experimental error. Unfortunately, insufficient quantity of the standard from Series I was available for further experimentation.

The objective of this experiment was to determine if the phosphorus in the pre-commercial defluorinated phosphate is indeed biologically available at about 70 to 75% of that in a sample of the experimental defluorinated phosphate used in Series I. The effects of decreasing the assay period to 2 weeks were also investigated.

MATERIALS AND METHODS

For this experiment, Large White turkeys were hatched and sexed at the Turkey Research Center. Males were randomized into 24 pens of one Petersime battery and females into 24 pens of another until each pen contained nine poults.

The composition of the basal diet used in this experiment is the same as that used in Series I (Table 1.1). The sources of phosphate evaluated were a defluorinated phosphate from an experimental source (TGG 4.25, or experimental defluorinated phosphate #1, from Series I) and a defluorinated phosphate from a pre-commercial source used in Experiment 1 as compared with dicalcium phosphate dihydrate, a laboratory product used in Experiment 1 (a J. T. Baker Chemical Company product, USP grade). Each source of phosphorus was added to the basal diet to supply .09, .12, .16, .22, .30, .40, .54, and .72% inorganic phosphorus forming a 3 × 8 factorial arrangement of diets. The experimental defluorinated phosphate contained 18.52% phosphorus, 31.78% calcium, and 4.33% sodium, the pre-commercial defluorinated phosphate contained 18.10% phosphorus, 32.72% calcium, and 4.00% sodium, and the dicalcium phosphate dihydrate standard contained 18.29% phosphorus and 23.65% calcium. These phosphorus supplements were added to the diet in place of ground limestone, salt, and ground yellow corn to hold calcium and sodium contents constant.

Each of the 24 diets was fed *ad libitum* to one pen of males and one pen of females during the 2-week experimental period. The poults were group-weighed at 1 day of age and at weekly intervals. Feed and mortality records were kept so that average feed consumption for the turkeys in each pen could be determined on a weekly basis.

At 2 weeks of age, all poults were killed and the middle toe from each foot excised from each bird at the second joint by the procedure of Fritz *et al*. (1969). The right toes of all birds within a pen were pooled, and the left toes of all birds within a pen were pooled. The 96 pooled toe samples were dried at 105 C for 18 hours and ashed at 600 C for 4 hours. The measurements of average body weight by pen and toe ash as percentage of dry toe were subjected to analyses of variance and to linear, asymptotic, and sigmoidal regression analyses. The reduction of the residual mean squares due to an increase in parameters in the regression equation, from linear to asymptotic to sigmoidal equation, was used to evaluate the improvement in degree of fit for each regression equation (Snedecor and Cochran, 1980).

RESULTS

Mortality of the poults fed diets containing the lowest two levels of phosphorus from the experimental defluorinated phosphate and the lowest three levels of phosphorus from the pre-commercial defluorinated phosphate was greater than 50% in contrast to only about 10% mortality from poults fed the diets with the same levels of phosphorus from dihydrate dicalcium phosphate (Table 3.10). Based on the mortality of the poults, the phosphorus in the two defluorinated phosphates is not as available as the phosphorus in the laboratory grade dicalcium phosphate dihydrate. The difference in mortality of poults fed diets containing the two defluorinated phosphates was not significant.

The average 0 to 2-week body weight gains of the poults fed the 24 diets are presented in Table 3.11, and the summary of the analyses of variance of the body weight gains by linear regression, asymptotic regression, and sigmoidal regression within sex is presented in Table 3.12. The asymptotic regression provided a much better fit ($P<.001$) than did the linear regression of the body weight gains on level of added phosphorus to the diets. The sigmoidal regression did not improve upon the asymptotic regression as a fit to these data. Thus, the asymptotic regression with three slopes, one for each phosphate, was used to determine the relative biological availability value of one phosphate to another. Plots of these data for males and females are presented in Figures 3.5 and 3.6, respectively.

The following two equations provided the best fit of body weight gains on levels of added phosphorus to the diet using the male and female poults, respectively:

$$Y = 45.1 + 184.3(1 - e^{-10.60X_1 - 6.65X_2 - 4.61X_3})$$

and

$$Y = 28.5 + 185.4(1 - e^{-10.87X_1 - 6.41X_2 - 4.48X_3})$$

where Y equals the 0 to 2-week body weight gain, and X_1, X_2, and X_3 the percent phosphorus added to the diet from dicalcium phosphate dihydrate, experimental defluorinated phosphate, and pre-commercial defluorinated phosphate, respectively. The ratio of 6.65 to 10.60 and 4.61 to 10.60 provided relative bioavailability values of 62.7 and 43.5%, respectively, for the experimental and pre-commercial defluorinated phosphates when using the body weight gains of males. Similarly, the value of 6.41 to 10.87 and 4.48 to 10.87 provided relative bioavailability values of 58.9 and 41.2%, respectively, for the experimental and pre-commercial defluorinated phosphates when using the body weight gains of females. The confidence limits about these ratios are presented in Table 3.13. The ratios of 4.61 to 6.65 and 4.48 to 6.41 provided relative bioavailability values of 69.4 and 69.9% for the phosphorus in the pre-commercial defluorinated phosphate to that in the experimental source from the data of the male and female, respectively. These values when pooled or averaged are significantly less than 100% indicating that the phosphorus in the pre-commercial defluorinated phosphate sample is less available than that in the experimental defluorinated phosphate sample.

The average ash contents of the dry toes of poults fed the 24 diets are presented in Table 3.14, and the summary of the analyses of variance of the toe ash measurements by linear regression, asymptotic regression, and sigmoidal regression within sex is presented in Table 3.15. The sigmoidal regression provided a much better fit ($P<.001$) than did the linear regression or the asymptotic regression on level of added phosphorus to the diet. Plots of these data for males and females and the equations of best fit are presented in Figures 3.7 and 3.8, respectively.

The following two equations provided the best fit to the data of toe ash percentage on level of added phosphorus to the diet using male and female poults, respectively:

$$Y = 3.7 + 7.5/(1 + e^{3.6 - 20.2X_1 - 12.8X_2 - 10.1X_3})$$

and

$$Y = 5.8 + 5.6/(1 + e^{8.2 - 43.0X_1 - 26.6X_2 - 21.6X_3})$$

where Y equals the percent toe ash of the dry toe of poults at 2 weeks of age, and X_1, X_2, and X_3 the percent phosphorus added to the diet from dicalcium phosphate dihydrate, experimental defluorinated phosphate, and pre-commercial defluorinated phosphate, respectively. The ratio of 12.8 to 20.2 and 10.1 to 20.2 provided relative bioavailability values of 63.6 and 50.1%, respectively, for the experimental and pre-commercial defluorinated phosphates when using toe ash measurements of males. Similarly, the ratios of 26.6 to 43.0 and 21.6 to 43.0 provided relative bioavailability values of 61.8 and 50.3%, respectively, for the experimental and pre-commercial defluorinated phosphates when using toe ash measurements of females. The confidence limits about these ratios are presented in Table 3.13. The ratios of 10.1 to 12.8 and of 21.6 to 26.6 provided relative bioavailability values of 78.8 and 81.2% for the phosphorus in the pre-commercial defluorinated phosphate to that in the experimental defluorinated phosphate from the data of males and females, respectively. Each of these values are significantly less than 100% indicating that the phosphate in the pre-commercial defluorinated phosphate is significantly less available than that in the experimental defluorinated phosphate.

DISCUSSION

Results of this experiment with phosphorus provide further evidence that the body weight gain of turkeys to 2 weeks of age followed an asymptotic function of level of phosphorus added to the diet. Because of this nonlinear relationship, the slope ratio assay regression as outlined by Finney (1978) is not applicable to the data from the phosphorus bioassay studies. Further, the use of the parallel line assay of Finney (1978) in these phosphorus experiments where body weight gain is considered a function of the log concentration of the added phosphorus is also not applicable unless several data points were removed from the analysis.

Results of toe ash measurements in this experiment followed a sigmoidal function of level of added phosphorus to the diet. Similar observations were made that the application of these toe ash measurements to the slope ratio assay using a multiple linear regression or to the parallel line assay using the logarithmic transformation of the concentration of added phosphorus was not appropriate. Thus, the application of the asymptotic equation to the body weight data and the sigmoidal equation to the toe ash data as a function of the level of added phosphorus to the diet provides a new procedure for use in evaluating the bioavailability of phosphorus from various sources in diets of poultry. In each case, any amount of added phosphorus to the diet from one source to that from another at a given constant ratio will provide equal response in body weight gain or in toe ash measurements. The reciprocal of these amounts provides a relative biological potency of the phosphorus from one source to that from another source.

Both the body weight gain and toe ash responses at the highest level of added phosphorus (.72% P) from the test phosphates were markedly lower than the responses at the .54% added phosphorus level. The responses from feeding the standard phosphate did not exhibit similar characteristics. The possible cause for the suppressed responses at the highest level of added phosphorus may be a chloride deficiency due to the sodium contribution without cloride from the phosphates in place of sodium chloride. The standard phosphate contained negligible amount of sodium, thus, at the highest level of phosphorus supplementation, the sodium chloride supplementation remained the same as that at the lowest phosphorus level. However, the regression analyses from data including and excluding the highest level of added phosphorus yielded the same results for the relative bioavailability and its 95% confidence limits due to the weight put on the data point at the extremes.

In Series I, the phosphorus in the experimental defluorinated phosphate was found to be 75% as available as that in the dicalcium phosphate dihydrate. In Experiment 1 of Series III, the phosphorus in a sample of pre-commercial defluorinated phosphate was found to be only 55% as available as that in the dicalcium phosphate dihydrate. Thus, it appeared that the phosphorus in the pre-commercial defluorinated phosphate was only 73% as available as that in the experimental defluorinated phosphate. In the current study, the same relationship was observed. On the average the phosphorus in the pre-commercial defluorinated phosphate was 75% ($46.3 \div 61.8$) as available as that in one of the samples of the experimental defluorinated phosphates. Using body weight gains, the ratio was 70%, and using the toe ash measurements, the ratio was 80%, the difference of which was not sufficient to be significant statistically.

In relationship to the phosphorus in dicalcium phosphate dihydrate, the phosphorus in both the experimental and in the pre-commercial defluorinated phosphate was significantly less available than that observed in the previous experiments. The relative bioavailability of the phosphorus in the experimental defluorinated phosphate was 75% in Series I and 62% in the current experiment. Different shipments (from different distributors and time period over 16 years) of the standard were used in the two experiments. Loss of water of hydration over time in the standard phosphate may account for part of the difference. Analyses showed that the dicalcium phosphate dihydrate sample used in Series I contained 18.6% phosphorus and the sample used in the current experiment contained 18.29% phosphorus. Based on molecular weight, a pure dicalcium phosphate dihydrate contains 17.996% phosphorus and an anhydrous dicalcium phosphate 22.76% phosphorus. A simple calculation yielded a blend of 87.4% dihydrate compound and 12.6% anhydrous compound, and 93.8% dihydrate and 6.2% anhydrous in the standards used in Series I and the current experiment, respectively. Dicalcium phosphate dihydrate was found to be 25 to 50% more available than the anhydrous form in chicks and poults up to 3 weeks of age (Griffith _et al_., 1966; Rucker _et al_., 1968). Using a value of 75% availability for phosphorus in the anhydrous dicalcium phosphate relative to that in the dihydrate form, the standards used in Series I and in the current experiment contained 96.85 and 98.45%

bioavailable phosphorus relative to a pure dihydrate compound, respectively, making the standard used in the current experiment 1.65% more available. Using a value of 50% availability, the bioavailable phosphorus contents were 93.7 and 96.9% for the anhydrous and the dihydrate forms, respectively, making the standard used in the current experiment 3.4% more available that that used in Series I. The loss of water of hydration in the standard samples could then account for 3 to 5% of the difference found in relative biological values obtained from the two experiments. Actual compounds present in the standard samples were more significant than the phosphorus and calcium contents when used in phosphorus bioassay.

The relative bioavailability of the phosphorus in the sample of pre-commercial defluorinated phosphate was 55% in Experiment 1 and 46% in the current experiment. Rucker _et al_. (1968) reported that phosphorus utilization in poults increased with age. Reduction of the experimental period from 3 to 2 weeks may account for some of the discrepancies though the 2-week body weight gain in Experiment 1 yielded a 49.8% relative biological value as compared with 43.5% in the male poults in this experiment. This difference may be accepted as being within the experimental error.

An important point of commercial significance has been observed. The sample of phosphorus from the production of the pre-commercial defluorinated phosphate is less biologically available than that from the experimental defluorinated phosphate production. Difference in bioavailability of phosphorus from various defluorinated phosphates exists. The cause for the difference is not known. However, some concern should be given to the condition of chemical reactions in the final steps of production of the defluorinated phosphate.

TABLE 3.10. Mortality during the 2-week experiment (Experiment 2)

Source of phosphorus	Added phosphorus, % .09	.12	.16	.22	.30	.40	.54	.72	Total
Standard $CaHPO_4 \cdot 2H_2O$	2[1]	1	3	1	0	0	1	0	10
Experimental defluorinated phosphate	13	13	8	4	4	4	0	0	42
Pre-commercial defluorinated phosphate	14	13	12	7	1	1	0	1	48

[1]Number of deaths per 18 poults (9 males and 9 females).

TABLE 3.11. Body weight gain from 0 to 2 weeks of age[1] (Experiment 2)

Source of phosphorus	Added phosphorus, % .09	.12	.16	.22	.30	.40	.54	.72
	(g)							
Standard $CaHPO_4 \cdot 2H_2O$	147.0[2]	173.5	175.2	210.8	236.2	225.0	221.0	240.0
Experimental defluorinated phosphate	140.4[3]	128.2[3]	146.4	177.2	201.0	207.7	225.9	164.5
Pre-commercial defluorinated phosphate	106.6[3]	104.4[3]	121.2[3]	153.4	182.0	193.6	219.3	205.7

[1]Difference required for significance between any two values equals 37.8 grams.

[2]Average of the surviving birds (average of males plus average of females divided by 2).

[3]Less than one-half of the poults survived on these treatments.

TABLE 3.12. Analysis of variance of body weight gains from linear, asymptotic, and sigmoidal regressions using data from males and females separately (Experiment 2)

Source	Degrees of freedom	Males: Sum of squares	Males: Mean squares	Females: Sum of squares	Females: Mean squares
Total about origin	24	884,535		744,514	
Mean	1	845,851		702,402	
Total about mean	23	38,684		42,112	
Linear regression	3	23,622		21,779	
Residual	20	15,062	753	20,233	1,012
Asymptotic regression	5	878,772		736,835	
Residual	19	5,763	303	7,679	404
Sigmoidal regression	6	878,827		737,706	
Residual	18	5,707	317	6,808	378
Reduction due to					
Asymptotic	1	9,299	9,299***	12,554	12,554***
Sigmoidal	1	56	56	871	871

***P<.001.

TABLE 3.13. Relative biological values of phosphorus from the experimental defluorinated phosphate and from the pre-commercial defluorinated phosphate to that from the dicalcium phosphate dihydrate as measured by body weight gains using the asymptotic regression analysis and by toe ash percentages using the sigmoidal regression analysis (Experiment 2)

	Experimental defluorinated phosphate		Pre-commercial defluorinated phosphate	
	Ratio	Confidence limits	Ratio	Confidence limits
	(%)	(%)	(%)	(%)
Asymptotic analysis using body weight gains				
Males	62.7	29.0-126.7	43.5	17.7- 84.7
Females	58.9	20.5-151.2	41.2	11.1-100.4
Sigmoidal analysis using toe ash percentages				
Males	63.6	48.9- 83.3	50.1	38.5- 66.0
Females	61.8	35.6- 90.7	50.3	31.9- 76.2
Average	61.8		46.3	

TABLE 3.14. Average ash content of the dry toes from poults at 2 weeks of age (Experiment 2)

Source of phosphorus	Added phosphorus, %							
	.09	.12	.16	.22	.30	.40	.54	.72
	(%)							
Standard $CaHPO_4 \cdot 2H_2O$	5.39[2]	5.98	6.92	9.08	11.22	11.75	11.21	12.05
Experimental defluorinated phosphate	4.73[3]	5.96[3]	5.28	6.14	7.83	10.73	11.18	10.01
Pre-commercial defluorinated phosphate	5.22[3]	5.19[3]	4.09[3]	5.58	6.57	8.69	11.07	10.41

[1]Difference required for significance between any two values equals 1.09%.

[2]Average of 4 determinations, 1 each from pooled right middle toes and from pooled left middle toes from male poults and from female poults.

[3]Less than one-half of the poults survived on these treatments.

TABLE 3.15. Analysis of variance of toe ash measurements from linear, asymptotic, and sigmoidal regressions using data from males and females separately (Experiment 2)

Source	Degrees of freedom	Males: Sum of squares	Males: Mean squares	Females: Sum of squares	Females: Mean squares
Total about origin	24	1,585.76		1,840.54	
Mean	1	1,403.01		1,683.63	
Total about mean	23	182.75		156.91	
Linear regression	3	143.59		114.39	
Residual	20	39.16	1.96	42.52	2.13
Asymptotic regression	5	1,573.81		1,814.23	
Residual	19	11.96	.63	26.31	1.38
Sigmoidal regression	6	1,579.52		1,831.03	
Residual	18	6.24	.35	9.51	.53
Reduction due to					
Asymptotic	1	27.20	27.20***	16.21	16.21**
Sigmoidal	1	5.72	5.72***	16.80	16.80***

**P<.01, ***P<.001.

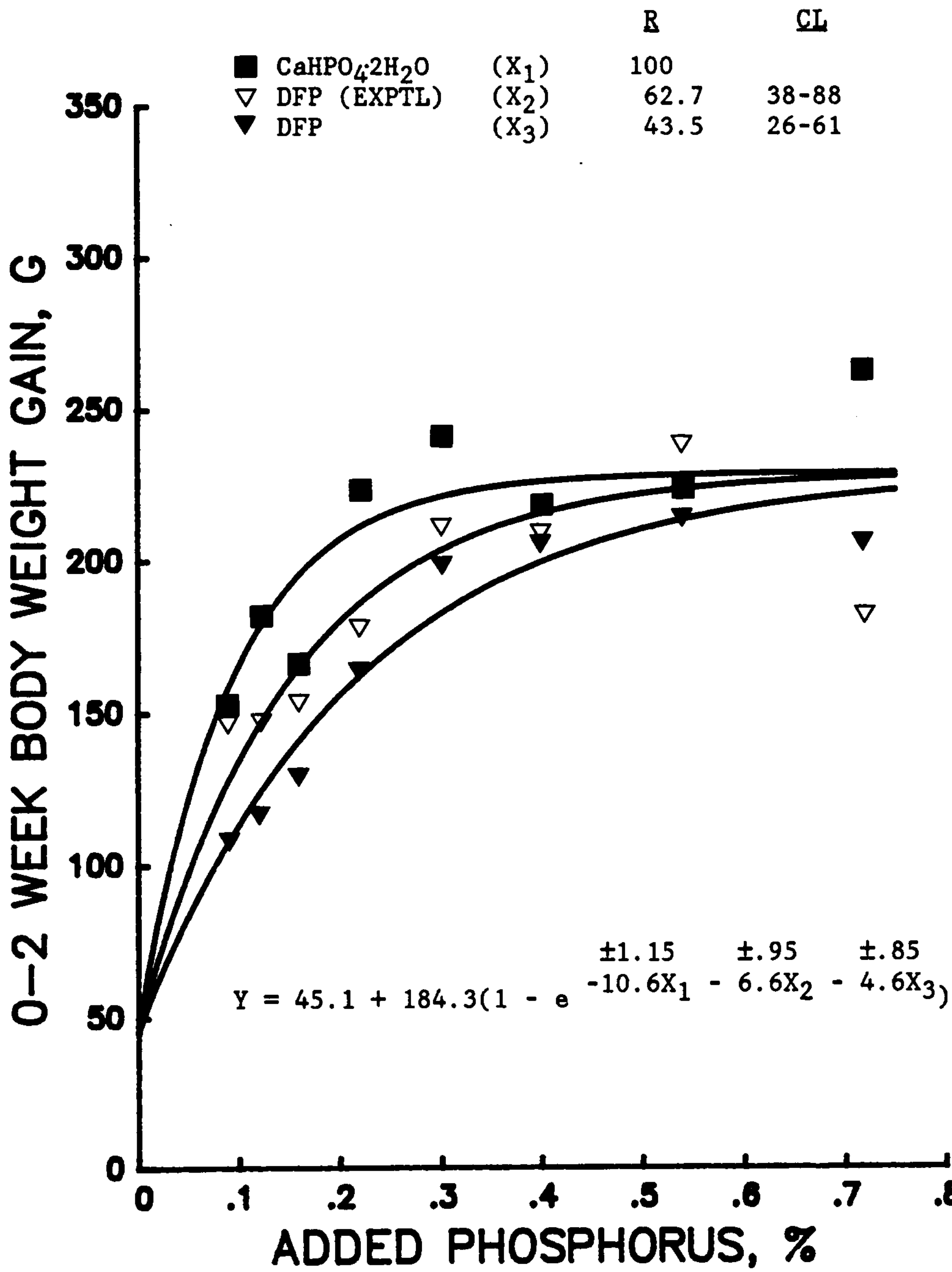

FIGURE 3.5. Plot of body weight gain of male turkeys from 0 to 2 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from three sources in Experiment 2. DFP=Defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from an experimental source.

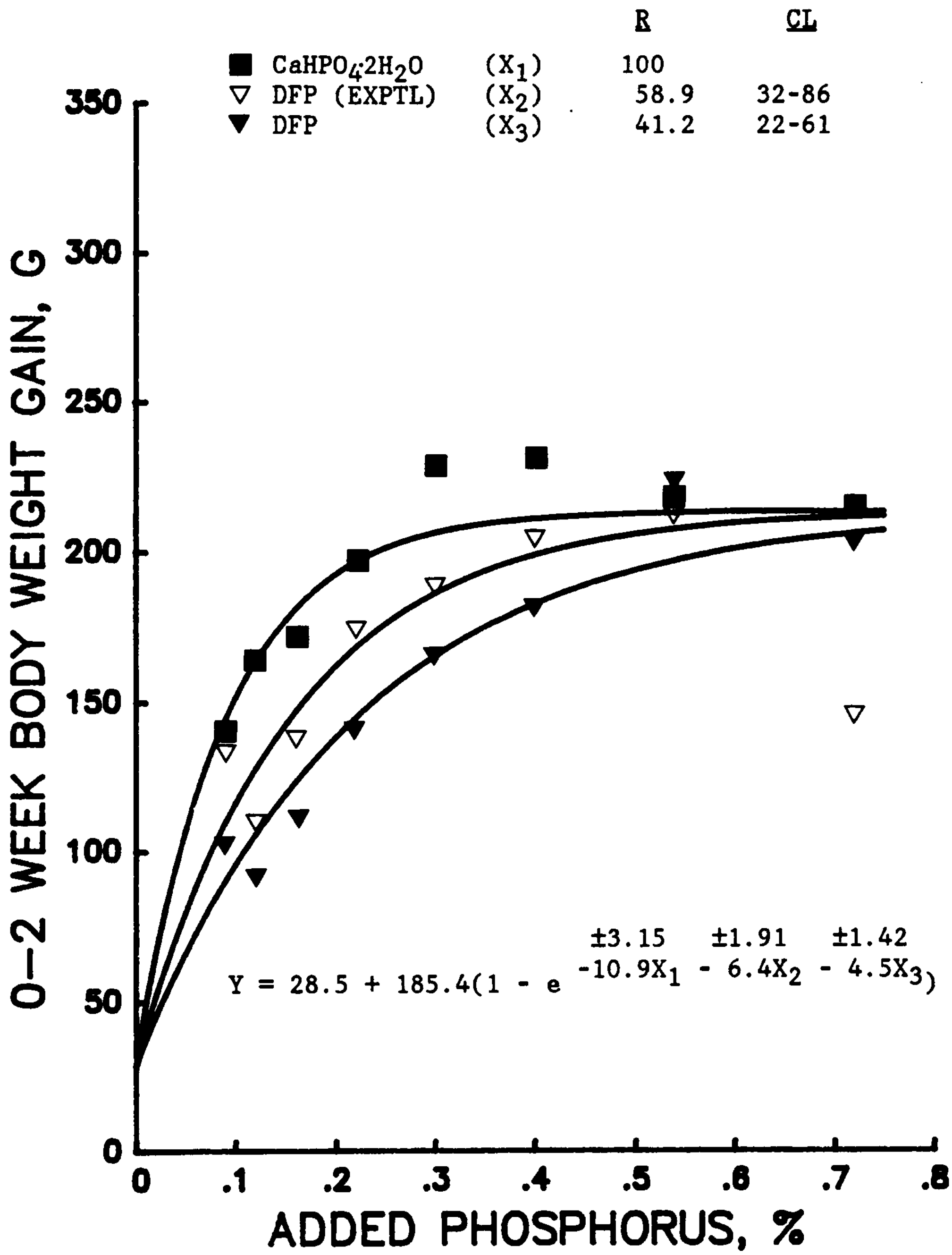

FIGURE 3.6. Plot of body weight gain of female turkeys from 0 to 2 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from three sources in Experiment 2. DFP=Defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from an experimental source.

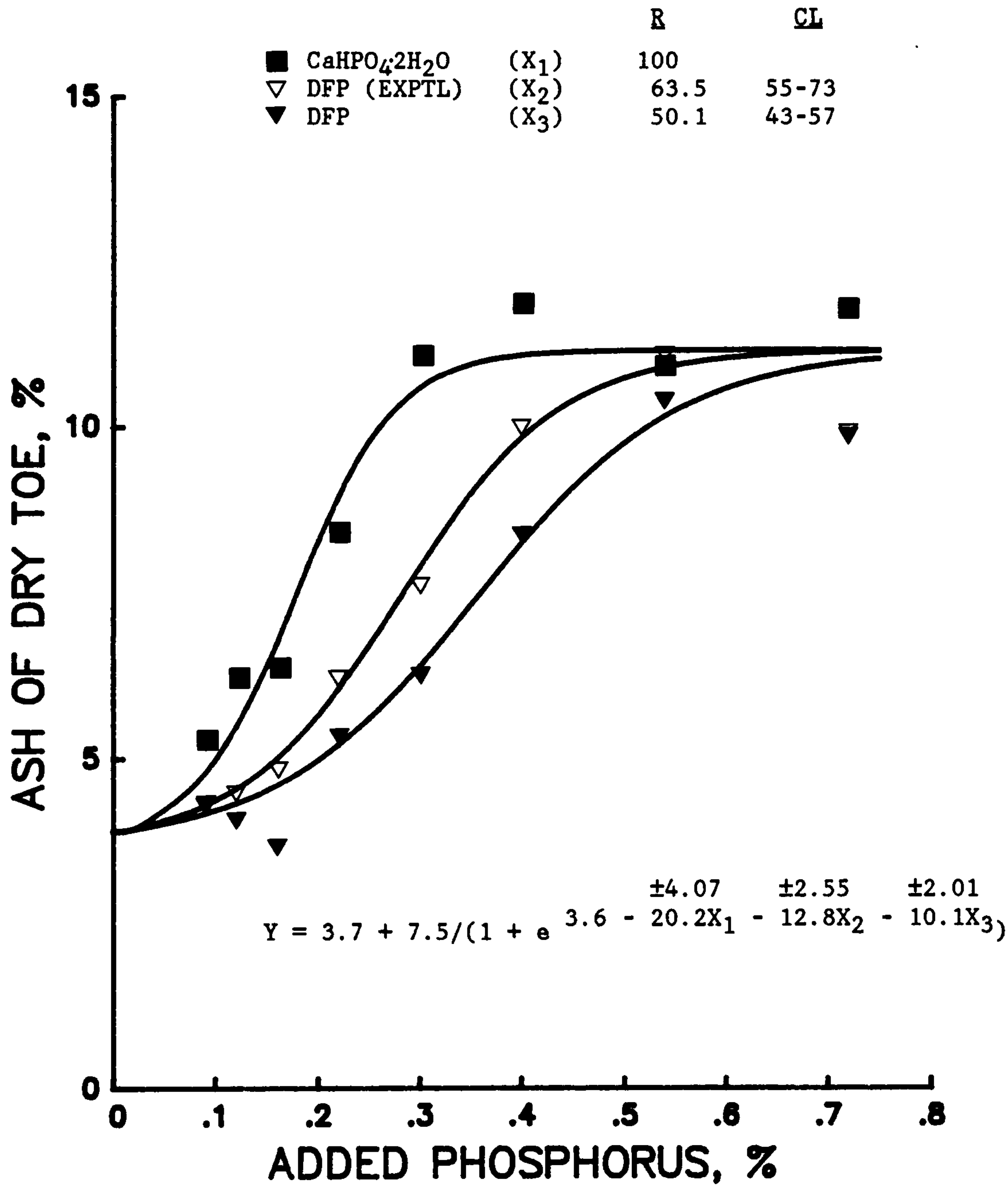

FIGURE 3.7. Plot of toe ash content of male turkeys at 2 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from three sources in Experiment 2. DFP=Defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from an experimental source.

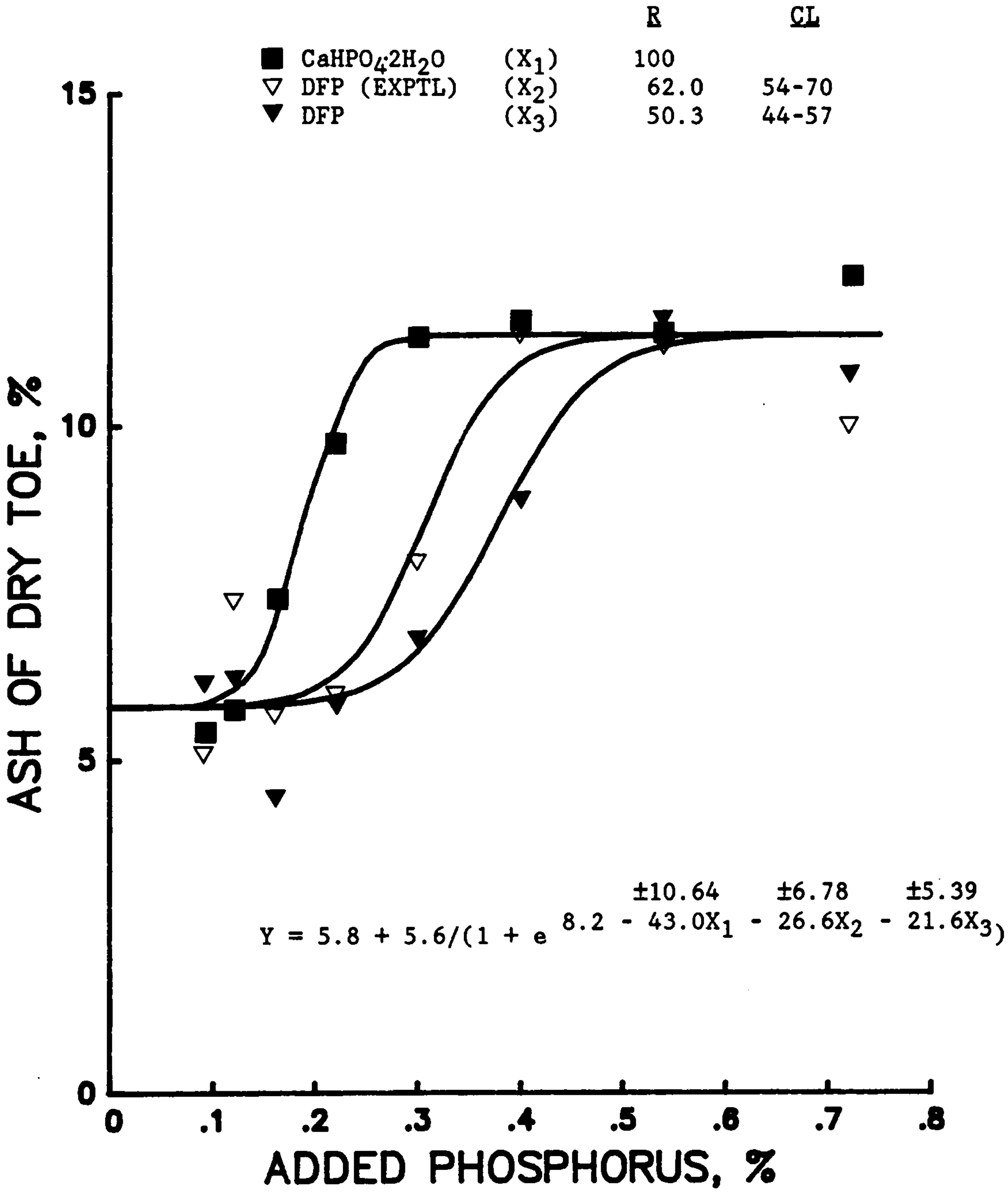

FIGURE 3.8. Plot of toe ash content of female turkeys at 2 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from three sources in Experiment 2. DFP=Defluorinated phosphate, DFP (EXPTL)=defluorinated phosphate from an experimental source.

EXPERIMENT 3

In Experiment 1, the phosphorus in a pre-commercial defluorinated phosphate was found to be 55% as available as that in dicalcium phosphate dihydrate in poults from 0 to 3 weeks of age. Phosphorus in the same defluorinated phosphate was found to be 46% as available as that in dicalcium phosphate dihydrate in Experiment 2 using poults from 0 to 2 weeks of age. Phosphorus in another commercial defluorinated phosphate source (Multifos) was found to be 70% as available as that in a different samples of dicalcium phosphate dihydrate in poults from 0 to 3 weeks of age (Series I).

Several researchers have reported a possible requirement for fluorine in growing chickens and turkeys (Merkley, 1981; Nahorniak et al., 1983). Nahorniak et al. (1983) suggested a 20 ppm supplementation of fluorine in the form of sodium fluoride for growing turkeys. They found the best 8-week growth response at 50 ppm but the response was not significantly different from supplementation levels of 12.5 and 25 ppm. Merkley (1981) found the effect of fluorine supplementation for chickens to be beneficial when fluorine was added to the diets of younger birds.

The objective of the present study was to determine the biological availability of phosphorus in various defluorinated phosphate samples including a high-fluorine defluorinated phosphate, a commercial defluorinated phosphate, and a sample of defluorinated phosphate used in Experiments 1 and 2 which was found to have a consistently lower relative bioavailability than other defluorinated phosphates tested. The high-fluorine defluorinated phosphate was included in the bioassay to determine the effect of underprocessing on the biological value of the phosphate product.

MATERIALS AND METHODS

The composition of the basal diet used in this experiment is presented in Table 1.1. The sources of phosphates evaluated in this experiment were three samples of defluorinated phosphates designated TG-DFP (a commercial defluorinated phosphate), TG-Fines (a non-commercial defluorinated phosphate), and TG-Hi-F (an under-processed defluorinated phosphate containing slightly higher fluorine content than the commercial product), one sample of pre-commercial TG-DFP from Experiment 1 (Test-DFP), and a commercial defluorinated phosphate (Multifos). A dicalcium phosphate dihydrate USP (J. T. Baker Chemical Company) was used as the standard. Compositions of the phosphates used in this experiment are presented in Table 3.16. Each source of phosphorus was added to the basal diet to supply .12, .15, .18, .21, .27, .36, .48, and .72% inorganic phosphorus forming a 6 × 8 factorial arrangement of diets. These phosphorus supplements were added to the diet in the place of ground limestone, sodium chloride, and ground yellow corn to hold calcium and sodium contents constants. In addition, potassium chloride was also added to diets containing the two highest levels of supplemented phosphorus for each phosphate source, except the dicalcium phosphate dihydrate, to maintain the chloride content at the same level as that in respective diet with .36% supplemented phosphorus. Potassium chloride was not required for diets containing the standard phosphate since the standard phosphate contained no sodium to replace sodium chloride, thus, resulting in a constant level of sodium chloride at all supplemented phosphorus levels.

Day-old Large White male turkeys obtained from Cuddy Hatchery, Harrisonburg, Virginia, were randomized into 96 pens until each pen contained 10 poults. Each of the 48 diets was fed *ad libitum* to two pens of poults during the 2-week experimental period. The poults were group-weighed at 1 day of age and at weekly intervals. Individual body weights were also recorded at 2 weeks of age. Feed and mortality records were kept so that average feed consumption and average feed efficiency for the turkeys in each pen could be determined on a weekly basis.

At 2 weeks of age, all poults were killed and the middle toe from each foot excised from each bird at the second joint by the procedure of Fritz *et al*. (1969). Pooled right toes and pooled left toes from birds within a pen were dried at 105 C for 18 hours and ashed at 600 C for 4 hours. The measurements of average body weight by diet and toe ash as percentage of dry toe by pen were subjected to analyses of variance and to linear, asymptotic, and sigmoidal regression analyses.

RESULTS

Mortality of the poults fed diets containing Test-DFP as their source of supplemental phosphorus during the 2-week experimental period was nearly double that of poults fed diets containing TG-DFP, TG-Hi-F, and Multifos as their sources of supplemental phosphorus which yielded the next highest mortality (Table 3.17). Based on the mortality of the poults, phosphorus in Test-DFP is the least available of all products evaluated. TG-DFP, TG-Hi-F, and Multifos with about the same mortality record are in the second group. The standard phosphate and TG-Fines are the two products with the highest phosphorus availability.

The average 2-week body weight gains of the poults fed the 48 diets were calculated on the pen basis and are presented in Table 3.18. The summary of the analyses of variance of the body weight gain by linear regression, asymptotic regression, and sigmoidal regression is presented in Table 3.19. The sigmoidal regression provided the best fit ($P<.05$) of all three regressions of the body weight gain on level of added phosphorus to the diet. Plot of these data is presented in Figure 3.9. The best-fit sigmoidal equation is as follows:

$$Y = 18.5 + 175.0/(1 + e^{1.6 - 18.47X_1 - 11.02X_2 - 15.37X_3 - 12.69X_4 - 8.13X_5 - 11.35X_6})$$

where Y equals the 2-week body weight, and X_1, X_2, X_3, X_4, X_5, and X_6 the percent phosphorus added to the diet from dicalcium phosphate dihydrate, TG-DFP, TG-Fines, TG-Hi-F, Test-DFP, and Multifos, respectively. Relative biological values using dicalcium phosphate dihydrate as a standard were 59.6, 83.2, 68.7, 44.0, and 61.5% for TG-DFP, TG-Fines, TG-Hi-F, Test-DFP, and Multifos, respectively. The confidence limits about these ratios are presented in Figure 3.9.

The average ash contents of the dry toes of poults fed the 48 diets are presented in Table 3.20, and the summary of the analyses of variance of the toe ash measurement by linear, asymptotic, and sigmoidal regressions is presented in Table 3.21. The sigmoidal regression provided the best fit ($P<.001$) to the data of the toe ash percentage on level of added phosphorus to the diet. Plot of these data is presented in Figure 3.10. The following equation provided the best fit to the data of the toe ash on level of added phosphorus to the diet:

$$Y = 5.2 + 6.5/(1 + e^{5.9 - 23.22X_1 - 17.36X_2 - 21.62X_3 - 18.39X_4 - 13.80X_5 - 17.13X_6})$$

where Y equals the 2-week toe ash percentage, and X_1, X_2, X_3, X_4, X_5, and X_6 the percent phosphorus added to the diet from dicalcium phosphate dihydrate, TG-DFP, TG-Fines, TG-Hi-F, Test-DFP, and Multifos, respectively. Relative biological values using dicalcium phosphate dihydrate as a standard were 74.8, 93.1, 79.2, 59.4, and 73.8% for TG-DFP, TG-Fines, TG-Hi-F, Test-DFP, and Multifos, respectively. The confidence limits about these ratios are presented in Figure 3.10.

A summary of relative potencies and confidence limits is presented in Table 3.22. When toe ash data were used, the relative bioavailability value of phosphorus in TG-Fines was not significantly different from that in the standard dicalcium phosphate dihydrate while phosphorus in each of the other phosphates is significantly less available than that in the standard. TG-Hi-F was found to be more available than TG-DFP when body weight gain data were used in the analyses but not significantly different from TG-DFP when toe ash data were used. Multifos was not significantly different from either TG-DFP or TG-Hi-F. Phosphorus in Test-DFP was significantly less available than that in all other phosphates evaluated.

DISCUSSION

Body weight gain and toe ash percentage at 2 weeks of age were shown in this experiment to follow a sigmoidal function of phosphorus added to the diet. The slope ratio assay (Finney, 1978) is not applicable to the data from the phosphorus bioassay studies due to the nonlinear relationship. Parallel line assay of Finney (1978) using logarithmic transformation of data is also not appropriate in these studies since several data points would have to be omitted from such an analysis.

In earlier studies, phosphorus in defluorinated phosphate samples from the same production plant was found to be 75% as available as that in dicalcium phosphate dihydrate standard, and phosphorus in Multifos was found to be 69.8% as available as that in the standard using poults from 0 to 3 weeks of age (Series I). In this experiment, phosphorus in the recent three samples of defluorinated phosphate from the same production plant, TG-DFP, TG-Fines, and TG-Hi-F, were found to be 67, 88, and 74% as available as that in the standard dicalcium phosphate dihydrate, respectively (Table 3.22). These values, except for TG-Fines, are similar to those reported earlier. Phosphorus in Multifos was found to be 68% as available as that in the standard which is also equal to the earlier reported value. Multifos sample used in this study was a different commercial sample from that used in Series I.

Several researchers have reported a possible advantageous effect of supplementing fluorine in poultry diet (Merkley, 1981; Nahorniak et al., 1983). Nahorniak et al. (1983) suggested a possible requirement of 20 ppm for fluorine as sodium fluoride in young growing turkeys, even though fluorine supplementation at 50 ppm was shown to give the best growth response at 8 weeks of age but not significantly different from growth response of those fed 12.5 and 25 ppm supplemented fluorine. Fluorine in sodium fluoride is considered to be the most available form of fluorine, and fluorine in defluorinated phosphate less available than that from sodium fluoride. Fluorine contents in TG-Hi-F and TG-Fines are higher than that in TG-DFP, and the highest level of fluorine supplementation with TG-Hi-F was 80 ppm in the diet with .72% supplemented phosphorus from TG-Hi-F. This level was well below the toxic level reported for young turkeys and was within the possible requirement level suggested by Nahorniak et al. (1983). Fluorine levels were also consistently higher in diets containing TG-Fines and TG-Hi-F than in respective diets containing TG-DFP. The difference in growth response and toe ash measurement may be due partly to fluorine content and/or availability of fluorine in various samples of defluorinated phosphate. The significantly higher availability of phosphorus in TG-Fines may be due in part to the finer particle size of the sample as compared with the other defluorinated phosphates. Exact compositions of the phosphate samples rather than the calcium and phosphorus contents may be the larger contributor to the differences in bioavailability between these samples.

Phosphorus in Test-DFP was reported to be 55 and 46% as available as that in the standard dicalcium phosphate dihydrate in poults 0 to 3 weeks of age and 0 to 2 weeks of age, respectively (Experiments 1 and 2, respectively). Phosphorus in Test-DFP was found to be 52% as available as that in the standard phosphate in this study. This result further confirmed the poor bioavailability value for Test-DFP.

The current TG-DFP was found to be equivalent to a commercial defluorinated phosphate, Multifos. An important finding is the superior availability of the TG-Fines which, if holds true when compared directly with commercial dicalcium and mono/dicalcium phosphates, may prove to have a higher biological value than dicalcium phosphate and in the same range as mono/dicalcium phosphate.

SUMMARY

Three experiments were conducted to determine the relative biological values of various defluorinated phosphate samples. A change in the standard dicalcium phosphate dihydrate sample from that used in Series I resulted in lower relative biological values for the phosphates under test. Phosphorus in the standard used in Series III was found to have a higher biological value than that used in Series I.

More levels of supplemented phosphorus were employed in the present experiments. A sigmoidal regression equation was found to provide a better-fit model for toe ash and tibia ash data. Body weight gain data as a response to supplemented phosphorus levels in the first two experiments exhibited asymptotic responses while a sigmoidal equation was found to provide a better fit for the body weight data in Experiment 3. Relative biological values and confidence limits calculated from both equations were equal.

Phosphorus in one sample of a pre-commercial defluorinated phosphate was found to be 55, 46, and 52% as available as that in the standard in Experiments 1, 2, and 3, respectively. The two lower values were derived from 2-week data rather than 3-week data in Experiment 1. The differences were within the range of experimental error.

Relative biological values of phosphorus in defluorinated phosphates produced from the same quarry rocks and processed in the same facility were found to be inconsistent, ranging from 51 to 88% with the commercial product at the time of the last experiment being 67% as available as the standard. Processing time and conditions have a direct effect on the

chemical compositions of the final products, e.g. the hydrated and anhydrous forms, the monocalcium and dicalcium phosphates, and the $CaCO_3$. An accurate measure of the chemical makeup of the phosphate compounds may explain the differences in bioavailability. Levels of fluorine in the defluorinated phosphates under test were not sufficient to establish any advantage of under-processing. The fluorine contents, however, did not exhibit any detrimental effects on bioavailability of the phosphorus in the tested defluorinated phosphates.

TABLE 3.16. Composition of phosphorus sources[1] (Experiment 3)

Source of phosphorus	Ca	P	Na	F
	(%)			
TG-DFP	33.87	18.30	4.60	.09
TG-Fines	33.42	18.22	4.66	.16
TG-Hi-F	33.68	18.10	4.96	.20
Test-DFP	33.72	18.10	4.00	.13
Multifos	32.00	18.00	4.90	.18
Standard	23.65	18.29	...	...

[1]All values were derived from chemical analyses (Association of Official Analytical Chemists, 1984), except for the Multifos whose values were from National Research Council (1984).

TABLE 3.17. Mortality during the 2-week experiment (Experiment 3)

Source of phosphorus	Rep	Added phosphorus, %								Total
		.12	.15	.18	.21	.27	.36	.48	.72	
TG-DFP	1	9[1]	5	4	1	0	0	0	0	19
	2	8	5	5	2	0	0	0	0	20
TG-Fines	1	6	0	0	0	0	0	0	2	8
	2	6	0	0	0	1	0	0	0	7
TG-Hi-F	1	8	4	0	6	0	0	0	0	18
	2	5	6	2	4	1	0	0	1	19
Test-DFP	1	8	9	9	9	4	1	0	0	40
	2	7	9	6	6	7	1	0	0	36
Multifos	1	8	4	3	2	0	0	0	0	17
	2	7	10	2	1	1	1	0	0	22
Standard	1	3	0	0	0	0	0	0	0	3
	2	1	1	1	0	0	0	0	0	3

[1]Number of deaths per 10 poults.

TABLE 3.18. Average body weight gain from 0 to 2 weeks of age (Experiment 3)

Source of phosphorus		Added phosphorus, %							
		.12	.15	.18	.21	.27	.36	.48	.72
	Rep	(g)							
TG-DFP	1	103.7	115.7	125.9	153.2	162.0	179.9	204.1	200.9
	2	61.1	113.5	128.4	135.4	129.2	176.9	185.8	189.0
TG-Fines	1	128.0	128.4	148.7	156.4	181.4	177.6	184.8	198.9
	2	127.0	139.2	149.8	165.9	182.7	180.5	185.5	204.8
TG-Hi-F	1	100.9	127.2	128.2	161.4	173.2	176.4	190.5	185.1
	2	97.8	112.0	128.6	145.4	176.8	186.2	201.6	189.0
Test-DFP	1	66.8	97.6	77.5	143.7	109.5	154.2	197.0	176.8
	2	92.2	83.8	119.4	97.0	107.9	165.8	197.6	194.7
Multifos	1	97.9	120.5	121.9	132.9	158.5	165.3	192.4	188.6
	2	94.6	...[1]	128.0	146.6	161.0	178.1	188.6	193.7
Standard	1	135.8	138.3	176.0	189.6	191.9	199.0	180.3	188.6
	2	126.3	155.8	164.0	174.4	187.4	184.4	203.4	213.2

[1]All poults died.

TABLE 3.19. Analysis of variance of body weight gain from linear, asymptotic, and sigmoidal regression (Experiment 3)

Source	Degrees of freedom	Sum of squares	Mean squares
Total about origin	95	2,385,231	
Mean	1	2,257,118	
Total about mean	94	128,113	
Linear regression	6	78,786	
Residual	88	49,327	561
Asymptotic regression	8	2,373,689	
Residual	87	11,541	133
Sigmoidal regression	9	2,374,338	
Residual	86	10,892	127
Reduction due to:			
Asymptotic	1	37,786	37,786***
Sigmoidal	1	649	649*

*P<.05, ***P<.001.

TABLE 3.20. Average ash of dry toe at 2 weeks of age (Experiment 3)

Source of phosphorus	Rep	.12	.15	.18	.21	.27	.36	.48	.72
		Added phosphorus, %							
		(%)							
TG-DFP	1	(9.12)[1]	5.60	5.46	5.54	6.69	9.08	11.18	11.32
	2	5.18	4.92	5.40	6.01	6.56	9.29	10.60	11.30
TG-Fines	1	5.06	5.69	5.94	6.60	8.42	11.06	11.34	12.20
	2	6.20	5.41	5.58	6.19	8.61	10.40	11.67	11.06
TG-Hi-F	1	5.94	4.76	5.74	6.10	7.08	9.49	11.98	11.64
	2	5.00	5.00	4.86	5.53	7.28	9.45	11.44	12.20
Test-DFP	1	(7.69)	5.01	6.50	7.87	5.64	6.90	10.10	11.42
	2	5.20	5.45	4.98	5.06	5.37	6.62	9.38	11.70
Multifos	1	6.16	5.18	5.86	4.66	7.13	8.17	11.40	12.28
	2	5.64	...[2]	5.12	5.02	7.58	9.09	10.98	10.98
Standard	1	4.94	5.66	6.28	7.28	8.49	11.89	11.66	11.77
	2	5.42	5.78	6.58	7.48	8.68	10.88	11.75	11.86

[1]Numbers in parentheses were not included in regression analyses.

[2]All poults died.

TABLE 3.21. Analysis of variance of toe ash from linear, asymptotic, and sigmoidal regression (Experiment 3)

Source	Degrees of freedom	Sum of squares	Mean squares
Total about origin	93	6,391.90	
Mean	1	5,771.25	
Total about mean	92	620.65	
Linear regression	6	516.71	
Residual	86	103.94	1.21
Asymptotic regression	8	6,328.45	
Residual	85	63.45	.75
Sigmoidal regression	9	6,358.59	
Residual	84	33.31	.40
Reduction due to:			
Asymptotic	1	40.49	40.49***
Sigmoidal	1	30.14	30.14***

***P<.001.

TABLE 3.22. Relative potency and confidence limit calculations[1] (Experiment 3)

Source of phosphorus	Body weight gain Error df = 86		Toe ash Error df = 84		Average
	R[2]	L[3] U[4]	R	L U	
	(%)	(%)	(%)	(%)	(%)
TG-DFP	59.6	56.9-62.4	74.8	68.6- 81.3	67.2
TG-Fines	83.2	79.4-87.2	93.1	85.7-101.1	88.2
TG-Hi-F	68.7	65.5-71.9	79.2	72.9- 86.1	74.0
Test-DFP	44.0	41.9-46.2	59.4	54.6- 64.7	51.7
Multifos	61.5	58.6-64.4	73.8	67.9- 80.2	67.6

[1]Relative potencies and confidence limits were calculated using sigmoidal regression equations,

[2]R = relative potency (%).

[3]L = lower confidence limit.

[4]U = upper confidence limit.

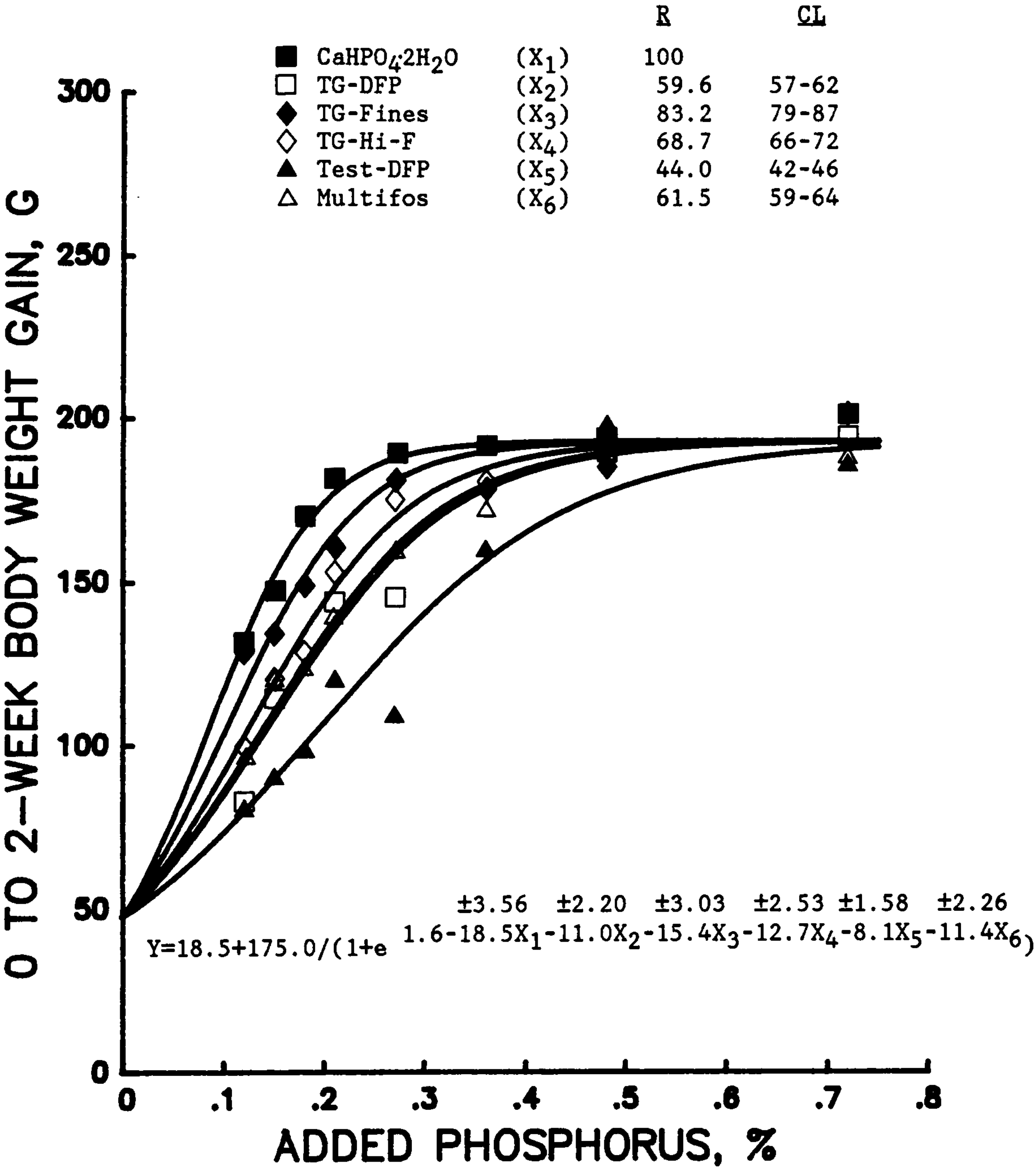

FIGURE 3.9. Plot of body weight gain of turkeys from 0 to 2 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from six sources in Experiment 3. TG-DFP and Multifos= commercial defluorinated phosphate, TG-Fines, TG-Hi-F and Test-DFP=defluorinated phosphate from experimental sources.

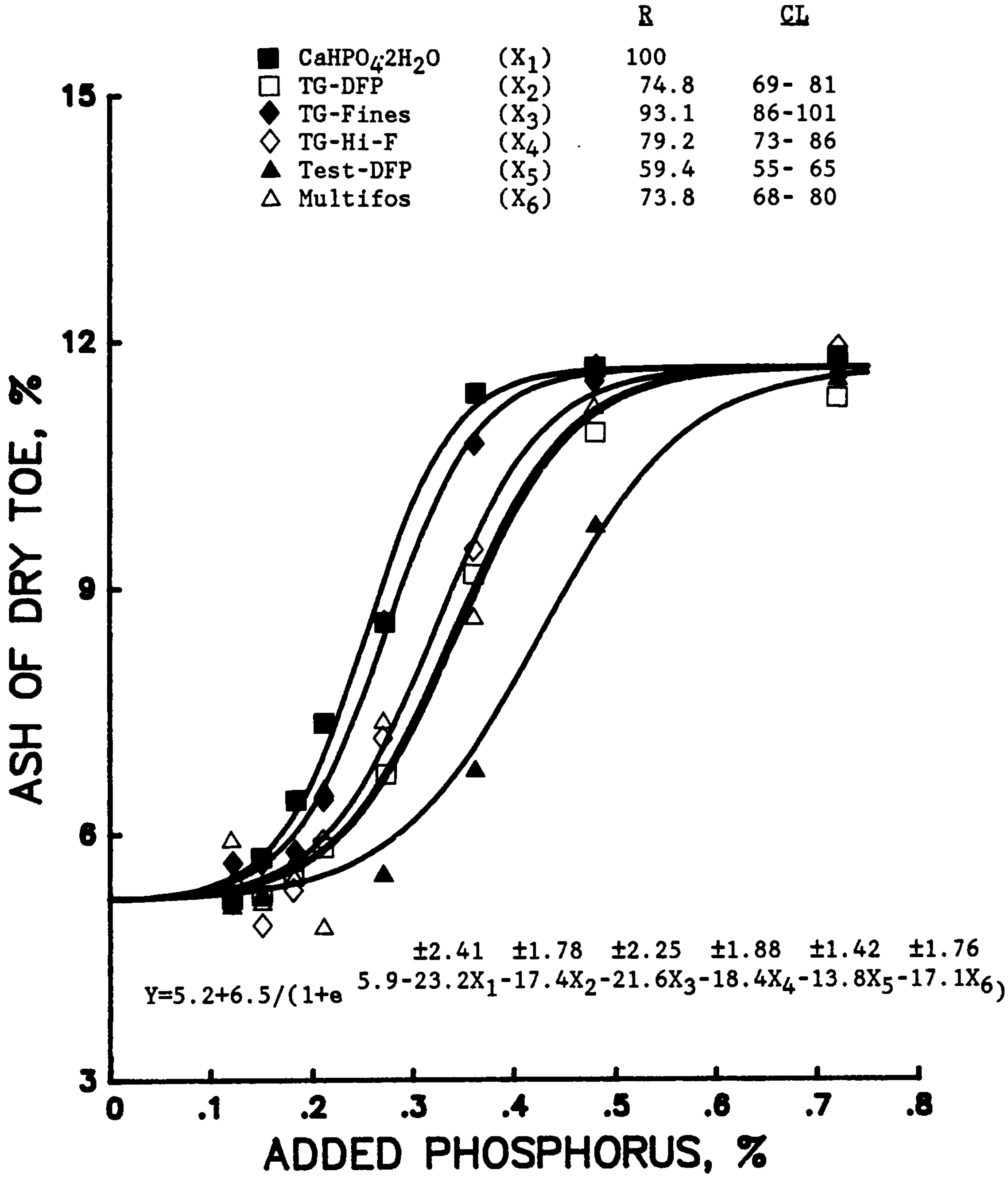

FIGURE 3.10. Plot of toe ash content of turkeys at 2 weeks of age on level of added phosphorus and relative biological values (R) with 95% confidence limits (CL) of phosphorus from six sources in Experiment 3. TG-DFP and Multifos=commercial defluorinated phosphate, TG-Fines, TG-Hi-F and Test-DFP= defluorinated phosphate from experimental sources.

LITERATURE CITED

Association of Official Analytical Chemists, 1984. Official Methods of Analysis. 14th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Baird, F. D., and M. J. MacMillan, 1942. Use of toes rather than tibiae in A.O.A.C. chick method of vitamin D determination. J. Assoc. Off. Agric. Chem. 25:518-524.

Chicco, C. F., C. B. Ammerman, P. A. van Walleghem, P. W. Waldroup, and R. H. Harms, 1967. Effects of varying dietary ratios of magnesium, calcium and phosphorus in growing chicks. Poult. Sci. 46:368-373.

Crowley, T. A., A. A. Kurnick, and B. L. Reid, 1963. Dietary phosphorus for laying hens. Poult. Sci. 42:758-765.

Damron, B. L., and R. H. Harms, 1968. A comparison of phosphorus assay techniques with chicks. 3. Development of a calcium standard curve for soft phosphate, defluorinated phosphate, and calcium phosphate. Poult. Sci. 47:1878-1883.

Damron, B. L., P. W. Waldroup, and R. H. Harms, 1967. Influence of diet composition on the utilization of soft phosphate in broiler diets. Poult. Sci. 46:1544-1549.

De Groote, G., 1983. Biological availability of phosphorus in feed phosphates for broilers. Pages 91-102 in: Fourth Eur. Symp. Poult. Nutr., Tours, France.

Dilworth, B. C., and E. J. Day, 1965. Effect of varying dietary calcium:phosphorus ratios on tibia and femur composition of the chick. Poult. Sci. 44:1474-1479.

Edwards, H. M., Jr., 1968. Chemical form and phosphorus availability. Pages 103-106 in: Proc. 1968 Georgia Nutr. Conf. Feed Manuf., Atlanta, GA.

Evans, R. J., and J. S. Carver, 1944. The toe ash as a measure of calcification in chicks. Poult. Sci. 23:351-352.

Evans, R. J., and J. L. St. John, 1944. Modification of A.O.A.C. chick method of vitamin D assay. J. Assoc. Off. Agric. Chem. 27:283-289.

Finney, D. J., 1978. Statistical Method in Biological Assay. 3rd ed. Charles Griffin & Company Ltd., London.

Fritz, J. C., 1969. Availability of mineral nutrients. Pages 1-15 in: Proc. 1969 Maryland Nutr. Conf. Feed Manuf., Washington, DC.

Fritz, J. C., and H. R. Halloran, 1943. Miscellaneous observations on the A.O.A.C. vitamin D assay. Poult. Sci. 22:314-322.

Fritz, J. C., and T. Roberts, 1968. Use of toe ash as a measure of calcification in the chick. J. Assoc. Off. Anal. Chem. 51:591-594.

Fritz, J. C., T. Roberts, J. W. Boehne, and E. L. Hove, 1969. Factors affecting the chick's requirement for phosphorus. Poult. Sci. 48:307-320.

Gillis, M. B., L. C. Norris, and G. F. Heuser, 1954. Studies on the biological value of inorganic phosphates. J. Nutr. 52:115-125.

Griffith, M., 1968. Influence of soybean products on the bone ash of chicks fed certain phosphorus-deficient purified diets. Poult. Sci. 47:765-771.

Griffith, M., and R. J. Young, 1967. Influence of dietary calcium, vitamin D_3 and fiber on the availability of phosphorus to turkey poults. Poult. Sci. 46:553-560.

Griffith, M., R. J. Young, and M. L. Scott, 1966. Influence of soybean meal on growth and phosphorus availability in turkey poults. Poult. Sci. 45:189-199.

Harms, R. H., and B. L. Damron, 1977. Phosphorus in broiler nutrition. Pages 1-89 in: Phosphorus in Poultry and Game Bird Nutrition. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Hurwitz, S., 1964. Estimation of net phosphorus utilization by the "slope" method. J. Nutr. 84:83-92.

Huyghebaert, G., G. De Groote, and L. Keppens, 1980. The relative biological availability of phosphorus in feed phosphates for broilers. Ann. Zootech. 29:245-263.

Johnson, S. R., 1942. Modifications in the chick vitamin D assay procedure. Poult. Sci. 21:329-332.

McGillivray, J. J., 1978. Biological availability of phosphorus sources. Pages 73-85 in: Proc. First Annu. Int. Minerals Conf., St. Petersburg Beach, FL.

Merkley, J. W., 1981. The effect of sodium fluoride on egg production, egg quality, and bone strength of caged layers. Poult. Sci. 60:771-776.

Motzok, I., 1968. Studies on phosphorus assay techniques. 1. The assay of soft phosphate with chicks. Poult. Sci. 47:967-974.

Motzok, I., D. Arthur, and H. D. Branion, 1956. Utilization of phosphorus from various phosphate supplements by chicks. Poult. Sci. 35:627-649.

Motzok, I., D. Arthur, and H. D. Branion, 1965. Factors affecting the utilization of calcium and phosphorus from soft phosphate by chicks. Poult. Sci. 44:1261-1270.

Motzok, I., D. Arthur, and S. J. Slinger, 1967. Utilization of calcium and phosphorus from soft phosphate by chicks. Poult. Sci. 46:985-991.

Nahorniak, N. A., P. E. Waibel, W. G. Olson, M. M. Walser, and H. E. Dziuk, 1983. Effect of dietary sodium fluoride on growth and bone development in growing turkeys. Poult. Sci. 62:2048-2055.

National Research Council, 1984. Nutrient Requirements of Poultry. 8th rev. ed. Natl. Acad. Press, Washington, DC.

Neagle, L. H., L. G. Blaylock, and J. H. Goihl, 1968. Calcium, phosphorus and vitamin D_3 levels and interactions in turkeys to 4 weeks of age. Poult. Sci. 47:174-180.

Nelson, T. S., 1967. The utilization of phytate phosphorus by poultry - a review. Poult. Sci. 46:862-871.

Nelson, T. S., L. W. Ferrara, and N. L. Storer, 1968. Phytate phosphorus content of feed ingredients derived from plants. Poult. Sci. 47:1372-1374.

Nelson, T. S., W. A. Hargus, N. Storer, and A. C. Walker, 1965. The influence of calcium on phosphorus utilization by chicks. Poult. Sci. 44:1508-1513.

Nelson, T. S., and H. T. Peeler, 1961. The availability of phosphorus from single and combined phosphates to chicks. Poult. Sci. 40:1321-1328.

Nelson, T. S., and A. C. Walker, 1964. The biological evaluation of phosphorus compounds. A summary. Poult. Sci. 43:94-98.

Noll, S. L., P. E. Waibel, R. D. Cook, and J. A. Witmer, 1984. Biopotency of methionine sources for young turkeys. Poult. Sci. 63:2458-2470.

Parks, J. R., 1970. Growth curves and the physiology of growth. I. Animals. Am. J. Physiol. 219:833-836.

Parks, J. R., 1971. Growth curves and the physiology of growth. IV. Effects of dietary methionine. Am. J. Physiol. 221:1845-1848.

Peeler, H. T., 1972. Biological availability of nutrients in feeds: availability of major mineral ions. J. Anim. Sci. 35:695-712.

Pensack, J. M., 1974. Biological availability of commercial feed phosphates. Poult. Sci. 53:143-148.

Ralston, M. L., and R. I. Jennrich, 1978. Dud, a derivative-free algorithm for nonlinear least squares. Technometrics 20:7-14.

Robbins, K. R., H. W. Norton, and D. H. Baker, 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710-1714.

Roland, D. A., Sr., 1985. Calcium and phosphorus in layer nutrition. Pages 1-110 in: NFIA Review on Calcium and Phosphorus in Animal Nutrition. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Rucker, R. B., H. E. Parker, and J. C. Rogler, 1968. Utilization of calcium and phosphorus from hydrous and anhydrous dicalcium phosphates. J. Nutr. 96:513-518.

SAS Institute Inc., 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc., Cary, NC.

Snedecor, G. W., and W. G. Cochran, 1980. Statistical Methods. 7th ed. The Iowa State Univ. Press, Ames, IA.

Sullivan, T. W., 1966. A triple response method for determining biological value of phosphorus sources with young turkeys. Poult. Sci. 45:1236-1245.

Sullivan, T. W., 1985. Calcium and phosphorus in turkey and game bird nutrition. Pages 1-66 in: NFIA Review on Calcium and Phosphorus in Animal Nutrition. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Twining, P. F., R. J. Lillie, E. J. Robel, and C. A. Denton, 1965. Calcium and phosphorus requirements of broiler chickens. Poult. Sci. 44:283-296.

Waibel, P. E., N. A. Nahorniak, H. E. Dziuk, M. M. Walser, and W. G. Olson, 1984. Bioavailability of phosphorus in commercial phosphate supplements in turkeys. Poult. Sci. 63:730-737.

Waldroup, P. W., 1985. Calcium and phosphorus in broiler nutrition. Pages 1-65 in: NFIA Literature Review on Calcium and Phosphorus in Animal Nutrition. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms, 1965a. The availability of phytic acid phosphorus for chicks. Poult. Sci. 44:880-886.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms, 1965b. A comparison of phosphorus assay techniques with chicks. Poult. Sci. 44:1086-1089.

Wilcox, R. A., C. W. Carlson, W. Kohlmeyer, and G. T. Gastler, 1955. The availability of phosphorus from different sources for poults fed practical-type diets. Poult. Sci. 34:1017-1023.

Willgeroth, G. B., J. L. Halpin, H. R. Halloran, and J. C. Fritz, 1944. Use of turkeys for assay of vitamin D. J. Assoc. Off. Agric. Chem. 27:289-295.

Yoshida, M., and H. Hoshii, 1977. Improvement of biological assay to determine available phosphorus with growing chicks. Jpn. Poult. Sci. 14:33-44.

Yoshida, M., and H. Hoshii, 1979. Monobasic calcium phosphate as a standard for bioassay of phosphorus availability. Jpn. Poult. Sci. 16:271-276.

Yoshida, M., and H. Hoshii, 1983. Relationship between ash contents of the tibia bone and the toe of chicks. Jpn. Poult. Sci. 20:51-54.

APPENDIX A

CONFIDENCE LIMIT CALCULATION MACRO

```
%MACRO CONFLMT (DATA=_LAST_,T=,STD=,TEST=,EDF=);
DATA WORK1; SET &DATA;
TITLE2 "CONFIDENCE LIMIT CALCULATIONS FOR STD OF &STD AND TEST OF &TEST";
    T = &T;
    IF T = . THEN DO;
    _ERROR_ = 1;
    PUT "THE PROGRAM CANNOT RUN WITHOUT A T VALUE";
    END;
   STD1 = "%STR(&STD)";
   TEST1 = "%STR(&TEST)";
   EDF = &EDF;
   IF EDF = . THEN DO;
    _ERROR_ = 1;
    PUT 'THE PROGRAM CANNOT EXECUTE WITH OUT AN ERROR DEGREES OF FREEDOM';
    END;
    EMS = _SSE_ / EDF;
    DATA FINAL; SET WORK1;
    IF _TYPE_ = 'FINAL';
     STD = &STD;
     TEST = &TEST;
       R = TEST/STD;
     DATA STD;  SET WORK1;
    IF _TYPE_ = 'COVB' AND
           _NAME_ = "%STR(&STD)" ;
         V11 = &STD/EMS;
    DATA TEST; SET WORK1;
    IF _TYPE_ = 'COVB' AND
           _NAME_ = "%STR(&TEST)";
         V22 = &TEST/EMS;
         V12 = &STD/EMS;
PROC PRINT DATA=FINAL;
PROC PRINT DATA=STD;
PROC PRINT DATA=TEST;
PROC SORT DATA=FINAL; BY _ITER_;
PROC SORT DATA=STD; BY _ITER_;
PROC SORT DATA=TEST; BY _ITER_;
DATA ALL; MERGE FINAL STD TEST; BY _ITER_;
PROC PRINT DATA=ALL;
DATA WORK1; SET ALL;
     LIST;
    EDF = 11;
    IF &T = . THEN T = 2.201;
    EMS = _SSE_ / EDF;
    S = SQRT(EMS);
    G = ( (T**2)*(S**2)*V11) /STD**2;
    A = G * V12 / V11;
    B = T  * S /STD;
    C = V22 - 2*R*V12 + (R**2)*(V11) ;
    D = V22 - V12**2 / V11;
    E = 1 - G;
    F = C - G * D;
```

```
    H = SQRT(F) ;
    I = B* H;
    J = R - A;
    K = J / E;
    L = I / E;
    RL = K - L;
    RU = K + L;
DATA _NULL_; SET _LAST_;
FILE PRINT ;
PUT @20 'CONFIDENCE LIMIT CALCULATIONS';
PUT / ;
PUT ' ERROR SUM OF SQUARES ' _SSE_;
PUT ' ERROR MEAN SQUARE    ' EMS;
PUT ' R VALUE =            '    R;
PUT ' S VALUE =            '   S;
PUT ' T VALUES =           '  T;
PUT ' STD VALUE =          ' STD;
PUT ' TEST VALUE =         ' TEST;
PUT ' V11 =                '   V11;
PUT ' V22 =                '   V22;
PUT ' V12 =                '   V12;
PUT ' G  =                 ' G;
PUT ' A  =                 ' A;
PUT ' B  =                 ' B;
PUT ' C  =                 ' C;
PUT ' D  =                 ' D;
PUT ' E  =                 ' E;
PUT ' F  =                 ' F;
PUT ' H  =                 ' H;
PUT ' I  =                 ' I;
PUT ' J  =                 ' J;
PUT ' K  =                 ' K;
PUT ' L  =                 ' L;
PUT ' LOWER LIMIT =        ' RL;
PUT ' UPPER LIMIT =        ' RU;
PROC PRINT DATA=WORK1;
%MEND CONFLMT;
```

APPENDIX B

APPENDIX B TABLE 1. Ash content of dry toes from poults at 3 weeks of age (Series III Experiment 1)

Added phosphorus	DFP[1]				Standard			
	Rep. 1		Rep. 2		Rep. 1		Rep. 2	
	left	right	left	right	left	right	left	right
(%)	(%)							
.09	1.85	6.37	...	...[2]	5.86	5.21	5.86	5.99
.11	5.48	4.47	3.87	3.89	6.50	6.27	7.25	6.76
.13	4.42	4.66	5.07	4.28	6.87	6.93	7.73	7.98
.15	3.76	5.09	6.29	5.54	7.38	7.60	8.35	8.14
.18	5.77	6.33	5.36	5.75	9.81	9.40	9.69	9.09
.21	7.44	7.63	6.48	6.39	10.82	10.68	10.29	10.20
.24	7.44	7.08	7.68	7.01	11.03	10.75	11.00	10.88
.27	8.18	8.06	7.07	8.38	12.02	11.68	10.89	10.89
.36	10.56	10.57	10.52	10.39	11.66	11.48	12.05	12.06
.45	11.76	11.40	11.12	10.66	11.89	12.10	11.68	11.43
.54	11.55	11.72	10.85	11.10	11.62	11.92	11.91	11.87
.72	11.10	11.15	11.29	11.42	12.05	11.94	12.44	12.42

[1]DFP = Defluorinated phosphate.

[2]All poults died.

APPENDIX B TABLE 2. Ash content of dry unextracted tibiae from poults at 3 weeks of age (Series III Experiment 1)

Added phosphorus		DFP[1]									
(%)	Rep.	(%)									
.09	1	32.21									
	2	...[2]									
.11	1	28.97									
	2	28.49	33.11								
.13	1	29.10	32.05	30.27							
	2	32.27	34.03								
.15	1	32.85	32.32								
	2	36.70	36.29	30.27							
.18	1	36.42	33.60	36.35	30.84	36.41	34.15				
	2	35.21	33.45	37.32	32.36						
.21	1	32.62	35.76	39.70	32.99						
	2	37.29	34.70	33.73	36.91	35.12					
.24	1	34.46	38.22	39.79	36.67	37.69	37.83	35.61			
	2	42.10	37.08	41.01	38.98	37.26	37.15	34.98	36.57	37.04	
.27	1	38.41	39.25	39.64	39.41	46.03	39.13	42.46	36.44		
	2	37.68	39.98	38.11	31.35	44.73	41.67	41.37	36.26	40.47	
.36	1	45.51	46.23	42.72	46.85	42.87	44.57	47.50	45.25		
	2	46.59	43.39	46.88	44.22	41.34	44.13	47.35	48.74	47.23	44.33
.45	1	47.79	46.37	49.80	47.85	48.02	47.04	46.27	45.17	47.58	46.08
	2	48.47	46.08	48.56	47.02	46.11	47.12	45.63	47.98	46.53	48.67
.54	1	50.04	47.98	48.21	46.18	48.06	50.10	48.62	50.06	48.29	48.31
	2	48.08	49.08	48.05	49.08	50.73	48.43	47.58	49.20	48.53	48.58
.72	1	44.25	49.06	47.04	47.68	51.50	48.72	50.98	48.24	48.25	48.37
	2	48.00	48.92	49.60	46.36	46.34	47.33	46.90	48.54	47.45	47.88

[1]DFP = Defluorinated phosphate.

[2]All poults died.

APPENDIX B TABLE 2. (cont.) Ash content of dry unextracted tibiae from poults at 3 weeks of age (Series III Experiment 1)

Added phosphorus		Standard									
(%)	Rep.	(%)									
.09	1	30.34	36.75	36.63	37.41	34.98	31.52				
	2	32.38	36.73	36.48	34.20	33.84	35.03				
.11	1	39.03	37.04	31.25	33.14	38.83	35.32	36.27	34.28	40.34	
	2	34.86	40.53	38.64	36.20	44.66					
.13	1	35.24	36.28	37.33	35.70	40.20	35.32	41.06	35.85		
	2	35.30	37.44	38.01	37.70	36.08	37.46	35.90	32.53		
.15	1	38.97	38.73	42.31	36.15	43.02	41.32	38.84	39.92	39.20	
	2	38.82	35.61	39.55	39.06	39.85	34.59	36.53	41.89	42.02	40.16
.18	1	42.59	42.81	41.58	44.55	42.51	40.90	45.18	41.67	43.10	45.26
	2	44.16	40.32	42.38	47.75	41.01	44.77	43.56	40.98	40.28	45.07
.21	1	43.99	43.87	44.45	44.41	45.51	44.54	44.01	44.30	43.25	44.76
	2	43.72	43.81	45.31	45.37	44.04	46.25	45.03	46.39	45.68	44.26
.24	1	44.67	46.59	46.39	44.46	47.29	46.12	46.15	45.09	45.09	45.79
	2	47.12	47.45	44.56	45.20	46.10	45.28	45.47	45.85	46.17	45.58
.27	1	48.99	47.01	46.78	49.67	47.41	49.24	45.74	49.14		
	2	47.14	47.62	48.30	47.45	47.07	50.75	46.43	48.28	46.51	49.71
.36	1	49.18	50.67	48.99	50.01	49.82	49.29	49.81	48.35	52.71	50.42
	2	50.81	51.18	49.82	49.73	49.81	51.27	51.28	48.78	50.69	50.68
.45	1	49.06	51.18	50.04	50.30	49.85	49.04	48.84	50.22	49.96	
	2	49.50	50.20	49.63	48.01	49.51	47.93	52.24	50.94	51.34	
.54	1	48.14	49.47	48.00	50.64	51.78	48.29	46.59	49.63	50.47	48.47
	2	47.90	51.42	48.31	50.12	51.21	48.88	49.82	51.15	50.27	48.99
.72	1	48.60	50.67	47.61	48.32	50.72	50.43	50.70	48.50	48.37	
	2	48.94	49.21	47.76	48.99	49.77	50.51	50.14	49.29	51.88	48.16

APPENDIX B TABLE 3. Body weight gain from 0 to 2 weeks of age (Series III Experiment 2)

Added phosphorus	Standard		DFP (EXPTL)[1]		DFP[2]	
	Male	Female	Male	Female	Male	Female
(%)	(g)					
.09	153.9	140.1	147.0	133.7	108.2	105.0
.12	183.1	163.9	146.3	110.1	116.9	91.8
.16	178.5	171.8	154.3	138.6	130.2	112.1
.22	223.8	197.7	179.1	175.2	164.9	141.8
.30	242.7	229.6	212.1	189.8	198.5	165.6
.40	219.1	231.0	210.1	205.3	204.8	182.4
.54	223.6	218.4	239.4	212.4	215.3	223.3
.72	263.6	216.3	182.8	146.2	207.4	204.0

[1]DFP (EXPTL) = Experimental defluorinated phosphate.

[2]DFP = Pre-commercial defluorinated phosphate.

APPENDIX B TABLE 4. Ash content of dry toes from poults at 2 weeks of age (Series III Experiment 2)

Added phosphorus	Standard		DFP (EXPTL)[1]		DFP[2]	
	Male	Female	Male	Female	Male	Female
(%)	(%)					
	Left middle toes					
.09	5.28	6.07	5.86	5.57	5.22	5.91
.12	6.17	6.11	4.92	7.39	3.79	7.40
.16	6.60	7.42	4.93	6.32	4.15	5.15
.22	8.42	9.27	6.61	6.18	5.95	6.43
.30	11.39	11.38	7.75	8.63	6.10	6.89
.40	12.17	11.93	10.08	11.60	8.47	9.01
.54	11.31	11.54	11.19	11.21	10.65	11.79
.72	11.74	12.16	10.34	10.15	10.61	10.68
	Right middle toes					
.09	5.34	4.86	2.83	4.64	3.31	6.46
.12	6.22	5.40	4.05	7.47	4.43	5.15
.16	6.22	7.42	4.81	5.07	3.28	3.77
.22	8.39	10.26	5.90	5.89	4.77	5.17
.30	10.82	11.27	7.55	7.39	6.45	6.84
.40	11.66	11.24	10.00	11.22	8.41	8.87
.54	10.65	11.33	11.02	11.30	10.28	11.55
.72	11.94	12.35	9.60	9.95	9.37	10.97

[1]DFP (EXPTL) = Experimental defluorinated phosphate.

[2]DFP = Pre-commercial defluorinated phosphate.

The vita has been removed from the scanned document