Improvement of Biological Assay to Determine Available Phosphorus with Growing Chicks

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In a series of multiple generation feeding experiments with yeast grown on n-paraffin¹⁾, it was observed that feed intake and growth rate of the chicks fed 15% of the yeast during growing stage, i. e., from 0 to 20 weeks of age, were 4% lower in average than those fed the control diet. Though the yeast is characteristic in high phosphorus and low calcium levels, no adjustment on dietary phosphorus level was done in the yeast diet mentioned above, resulting in slightly higher phosphorus level than the level recommended in Feeding Standard²⁾ and in unbalance of calcium and phosphorus levels. It was confirmed later³⁾ that one of the main reasons of lowered appetite and of retarded growth observed on the yeast diet was high and unbalanced phosphorus level in the diet. Feed intake and growth rate of chicks fed an yeast diet, of which phosphorus and calcium levels as well as protein and energy levels were carefully adjusted to be identical to those of the control diet by omitting dibasic calcium phosphate, were almost exactly identical to those of chicks fed the control diet³⁾. The findings indicate clearly that phosphorus in the yeast must be as available by chicks as phosphorus in dibasic calcium phosphate is. To confirm high availability of phosphorus in the yeast by chicks, it is necessary to establish a biological procedure for estimating availability of phosphorus by chicks.

Most simple way to evaluate biological availability of phosphorus is to compare growth rate and bone ash content of chicks fed test material to those fed the standard phosphorus source. BARUAH *et al.*⁴⁾ and HIJIKURO *et al.*⁵⁾ showed relatively the response of the chicks on the test material by an index taking the response of the chicks on the standard as 100.

GILLIS *et al.*⁶⁾ first tried to determine quantitatively the availability of phosphorus, based on the linear relationship between dietary level of phosphorus and bone ash content. In the procedure, phosphorus equivalent of the test material is read on the dose-response line against the response of the chicks on the test material. DAMRON and HARMS⁷⁾ applied this procedure and found large variation of availability of phosphorus in the test material, mainly due to errors in obtaining standard dose-response line and in reading availability on the standard line, although they did not discuss about the error in the estimation. AMMERMAN *et al.*⁸⁾ estimated the regression coefficient between supplemental phosphorus level and bone ash content, and the slopes were compared. SUMMERS *et al.*⁹⁾ took logarithm of supplemental phosphorus and found that log dose-response curve linear. Accordingly, availability of phosphorus in test materials was estimated by parallel line assay. NELSON and PEELER¹⁰⁾ also found that log dose-response

curve linear within wider range than dose-response curve is. The parallel line assay based on log dose-response lines on the standard and test materials was reviewed by NELSON and WALKER¹¹⁾, and used by DILWORTH and DAY¹²⁾, SPANDORF and LEONG¹³⁾, MOTZOK¹⁴⁾ and NELSON *et al.*¹⁵⁾. WALDROUP *et al.*¹⁶⁾ compared the assay procedures based on log dose and on dose itself and found that similar availability was obtained by the two assay procedure.

HURWITZ¹⁷⁾ proposed different way to evaluate biological value based on the net phosphorus utilization, *i. e.*, phosphorus retention, per unit of phosphorus taken.

In most of the assays, experimental diets were fed for 3 or 4 weeks^{4,6,7,9-16,18-24)}. AMMERMAN *et al.*⁸⁾ proposed a rapid method, in which test period is 6 days after 4 days of depletion period, but later, AMMERMAN *et al.*³⁵⁾ reported that the rapid method was inferior to the usual assay procedure of 4 weeks of period.

In supplementing graded levels of standard phosphorus, dietary calcium level was kept constant at 1%, or dietary calcium and phosphorus levels were so adjusted to keep the ratio constant at 2 : 1 in majority of the papers cited in this paper. WALDROUP *et al.*¹⁶⁾ concluded that sliding optimum calcium level for various phosphorus levels is preferable to the other two processes, *i. e.*, keeping calcium level constant at 1% or keeping calcium and phosphorus ratio constant at 2 : 1, although tibia ash content determined after feeding the diets prepared by one of above 3 processes seemed to be close each other. As for the ratio of dietary calcium and phosphorus levels, it has been reported that the change in the ratio has little effect on the assay result when the ratio is within 2 : 1 to 1 : 1^{14,29)}.

Tibia ash content has been used as criteria for biological availability of dietary phosphorus in most of the assay technique. In one report²⁷⁾, toe ash content, which is much simpler in determination than tibia ash content is and which is used as criteria in vitamin D assay with chicks³⁶⁾, was used as criteria for phosphorus assay. Ash content of lower beak was reported to be less sensitive to the change in dietary level of available phosphorus than ash content in tibia is³⁵⁾.

In this paper, are presented results of studies on the suitable term of experiment, on the effectiveness of toe ash content as criteria and on effect of change in dietary calcium levels. A simplified and rapid biological assay procedure with high accuracy is recommended, which is a slope ratio assay based on linear dose-response relationship.

Experimental

Four kinds of experiments were conducted. In the first kind of experiment, 79 male White Leghorn chicks and 86 male and 85 female meat-type cross-bred chicks (White Cornish x White Plymouth Rock) of one-day-old were fed low phosphorus standard diet for 14 days. Twenty chicks each were planned to sacrifice at 8, 10, 12 and 14 days after the start of feeding and to determine ash content of the tibia and toe.

In the second kind of experiment, 340 White Leghorn male chicks of 7-day-old, which were raised on the restricted feeding program³⁷⁾, were divided into two groups

of equal number and fed the standard diets containing either 0 or 0.3% of added phosphorus. Twenty chicks each were randomly selected and sacrificed at 6, 8, 10, 12, 14, 21, 28 and 35 days after the start of the experiment. Ash content of the tibia and toe of 5 chicks as a group were determined. Rest of the chicks were discarded at 35 days. Another 20 chicks of the same group were sacrificed at the beginning of the experiment to determine ash contents of the tibia and toe.

In the third kind of experiment, 4 experiments, Expts. 505, 506, 507 and 511 were conducted, in which availability of phosphorus of feed grade dibasic calcium phosphate, fish meal and yellow corn were determined biologically, using the tibia and toe ash contents as criteria. In Expts. 506, 507 and 511, fifteen White Leghorn male chicks each, which were raised on the restricted feeding program⁸⁷⁾ for one week, were fed the standard and test diets of various phosphorus levels for 10 days. In Expt. 505, 10 chicks each of meat-type cross-bred chicks of both sexes were treated as White Leghorn chicks mentioned above, except the chicks were sacrificed at 8 days because the ricket was observed among the chicks fed the standard diet containing no additional phosphorus. In each experiment, ash content of the tibia and toe of 5 chicks as a group were determined.

In the fourth experiment, effect of dietary calcium level on the response of the chicks to phosphorus level was studied. Duplicated lots of 5 chicks each of 6-day-old White Leghorn male, pre-fed on the restricted feeding program⁸⁷⁾, were fed one of 9 diets, containing 3 levels of calcium, *i. e.*, 0.8, 1.0 and 1.2%, and 3 levels of added phosphorus, *i. e.*, 0, 0.15 and 0.3%, in combination for 8 days. The chicks were sacrificed to determine ash content of the tibia and toe.

The chicks in these experiments were reared in an electrically-heated wire-floor battery breeder. Feed and water were given in free access during the experimental period.

Composition of standard and test diets are presented in Table 1. The standard diets contained 3.00 kcal of metabolizable energy/g diet, 20% of crude protein and 1.0% of calcium and various levels of added phosphorus from calcium phosphate. As mentioned in Footnote 1 in Table 1, the standard diets were designated by added phosphorus. Only other phosphorus source in the standard diets was soybean meal, which supplied 0.3% of phosphorus.

To mix various phosphorus sources, dietary cornstarch, cellulose and calcium carbonate were adjusted to keep levels of metabolizable energy and calcium constant. Feed grade dibasic calcium phosphate was added to supply 0.3% of phosphorus. Fish meal was added to supply 0.184% of phosphorus. To mix samples of low phosphorus centnet such as cereals, 49.52% of cereal was mixed with constant part II, of which soybean oil and vitamin B mixture were reduced. Standard and test diets containing various levels of phosphorus were prepared by mixing P-0 standard diet and the diet containing standard or test sources shown in Table 1. For example, 1:1 mixture of P-0 and P-0.3 diets gives P-0.15 standard diet.

In Table 2, is presented chemical composition of standard and test materials, based on which the composition of the diets presented in Table 1 was decided. Metabolizable

Table 1. Composition of diet

Ingredient	Standard ¹⁾		Calcium phosphate dibasic ²⁾	Fish meal	Yellow corn
	P-0	P-0.3			
	%	%	%	%	%
Constant part I ³⁾	53.4	53.4	53.4	53.4	—
Constant part II ⁴⁾	—	—	—	—	48.90
Cornstarch	37.6	37.6	37.6	32.22	—
Cellulose	7.32	7.22	6.62	6.67	—
Calcium carbonate	1.68	0.07	0.69	0.71	1.58
Phosphorus source	—	1.71 ⁵⁾	1.69	7.0	49.52

1) Designated by added phosphorus from calcium phosphate. For example, P-0 and P-0.3 contains 0 and 0.3% of added phosphorus, respectively.

2) Calcium phosphate, dibasic, feed grade.

3) Composed of 44.6% of soybean meal, 0.2% of DL-methionine, 6% of soybean oil, 0.5% of sodium chloride, 0.1% of trace mineral mixture⁶⁾, 1.5% of vitamin B mixture⁷⁾ and 0.5% of vitamin A and D supplement⁸⁾.

4) Composed of 44.6% of soybean meal, 0.2% of DL-methionine, 2% of soybean oil, 0.5% of sodium chloride, 0.1% of trace mineral mixture⁶⁾, 1.0% of vitamin B mixture⁷⁾ and 0.5% of vitamin A and D supplement⁸⁾.

5) Calcium phosphate, tribasic, reagent grade.

6) Composed of 67.28% of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 29.91% of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.43% of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.29% of $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.09% of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$.

7) One g of vitamin B mixture contained 1.2 mg of thiamine-HCl, 1.2 mg of riboflavin, 10 mg of niacin, 6 mg of pyridoxine-HCl, 4 mg of calcium pantothenate, 170 mg of choline chloride, 100 mg of inositol, 0.5 mg of folic acid, 2 mg of dl- α -tocopheryl acetate, 10 mg of para-aminobenzoic acid, 0.05 mg of biotin, 0.06 mg of menaquinone and 5 μg of vitamin B₁₂.

8) One g of vitamin A and D supplement contained 10,000 I.U. of vitamin A palmitate and 2,000 I.C.U. of vitamin D₃.

Table 2. Chemical composition of standard materials and samples

Sample	Moisture	Crude protein	Calcium	Phosphorus
	%	%	%	%
Calcium phosphate, tribasic	3.38	— ¹⁾	36.7	17.5
Calcium phosphate, dibasic	—	—	22.8	17.7
Calcium carbonte	—	—	39.1	—
Fish meal	9.63	58.9	5.42	2.63
Yellow corn	14.36	8.93	0.01	0.26
Soybean meal	11.51	44.4	0.32	0.67

1) Not determined.

energy of cornstarch, soybean oil and cellulose were estimated as 3.52, 9.21 and 0 kcal/g. Nine diets used in the fourth experiment were prepared by adjusting calcium phosphate, calcium carbonate, cornstarch and cellulose.

Moisture and crude protein in the standard and test materials were determined by usual procedure for feed analysis³⁸⁾. Their calcium and phosphorus were determined by atomic absorptien analysis and colormetry with vanadomolybdenum reagent, respectively. after extracting the sample itself or the ash of the sample with diluted hydrochloric acid³⁸⁾. Ash content of the tibia and toe was determined as described in Official Meth-

ods of Analysis of the Association of Official Analytical Chemist³⁶⁾.

Slope ratio assay of ash content of the tibia and toe was done as described in the previous paper³⁹⁾.

Fig. 1. Cumulative change in incidence of ricket.
(Figures in parentheses are total number of chicks tested)

Fig. 2. Change in body weight and ash content of tibia and toe of 7-day-old chicks.
(Vertical line indicates standard deviation)

Results

One-day-old chicks when fed the P-0 diet showed symptoms of ricket. As shown in Fig. 1, clear breed difference in the susceptibility to the ricket was observed, while sex difference was not. Meat-type cross-bred chicks were suffered from ricket at as early as the fourth day on the diet, and the number of chicks suffering ricket increased rapidly to reach almost 90% at the 12th day. Incidence of ricket of egg-type chicks was only 33% at the 14th day. Difference in incidence of ricket between two breeds of chicks was significant at 1% level by χ^2 -test at the 12th day.

In Fig. 2, change in body weight and ash content in the tibia and toe of 7-day-old chicks fed either P-0 or P-0.3 diet are presented. As expected previously, growth of the chicks fed P-0.3 diet was faster than that fed P-0 diet. Although not presented in Fig. 2, feed intake of the chicks increased smoothly, and that of the chicks fed P-0.3 diet was more than that fed P-0 diet. The difference in body weight and feed intake between the chicks fed two diets was not so large until after 14 days in the experiment. Ash content of the tibia of the chicks fed P-0 diet and of toe of the chicks decreased after feeding the diets and reached the lowest at 8~12 days, then increased gradually. Ash content of the tibia of the chicks fed P-0.3 diet increased gradually, but was almost the same during 8~12 days after feeding the diet. The trends of the change in ash content of the chicks fed either P-0 or P-0.3 diet were almost parallel, result-

Fig. 3. Change in tibia and toe ash content of chicks fed graded levels of phosphorus (Expts. 505 and 507)

Fig. 5. Change in tibia and toe ash content of chicks fed graded levels of phosphorus (Expt. 506)

Fig. 4. Change in body weight and feed intake of chicks fed graded levels of phosphorus.

Fig. 6. Changes in toe ash content of chicks fed graded levels of phosphorus (Expt. 511)

ting in parallel lines as shown in Fig. 2.

Responses of the chicks fed graded levels of phosphorus in Expts. 505, 506, 507 and 511 are presented in Figs. 3, 4, 5 and 6. As shown in Figs. 3, 5 and 6, toe ash content of the chicks was in proportion to the dietary levels of added phosphorus from 0 to 0.3% while tibia ash content was not always in proportion. Especially, tibia ash content at the highest added phosphorus level was lower than that expected from ash contents at lower levels. The linear relationship was observed in toe ash content of both egg-type and meat-type chicks as shown in Fig. 3. In contrast, change in body weight gain and feed intake of the chicks fed graded levels of phosphorus varied as shown in Fig. 4, although the trend of increasing linearly with increase of dietary phosphorus levels was observed. The experimental period of 10 days was too short to obtain clear-cut linear relationship between body weight or feed intake and dietary phosphorus levels.

Availability and its 95% fiducial interval of feed-grade dibasic calcium phosphate were estimated from the toe ash content in Fig. 3 by 9-point slope ratio assay to be 99% and 115~84%, respectively. The corresponding values estimated from the tibia ash content of chicks fed less than or equal to 0.225% of added phosphorus by 7-point slope ratio assay were 103% and 117~90%, respectively.

Availability and its 95% fiducial interval of phosphorus in fish meal were estimated from the toe ash content of the chicks shown in Fig. 5 by 5-point slope ratio assay to be 109% and 127~90%, respectively. In this assay, one unit of added phosphorus from fish meal was 0.092%, while that from standard calcium phosphate was 0.15%. The ratio, $0.092/0.15=0.61$, was chosen to make the fiducial interval almost the narrowest³⁹⁾.

Availability and its 95% fiducial interval of phosphorus in yellow corn were estimated from the toe ash content shown in Fig. 6 by 5-point slope ratio assay to be 0% and 33~-31%, respectively.

Body weight gain and ash content in the tibia and toe of the chicks fed the diet

Table 3. Body weight gain, tibia ash and toe ash of chicks fed diets of various levels of calcium and phosphorus

Dietary level		Body weight gain	Tibia ash	Toe ash
Ca	P			
%	%	g/day	%	%
0.8	0	5.25	33.36	9.75
"	0.15	7.32	38.98	11.51
"	0.3	8.00	42.24	12.54
1.0	0	5.27	33.79	9.90
"	0.15	6.68	38.40	11.16
"	0.3	8.24	42.82	13.19
1.2	0	4.05	34.61	10.51
"	0.15	5.95	36.19	10.63
"	0.3	7.63	39.74	11.63

Table 4. Analysis of variance of effect of dietary calcium and phosphorus levels

Factor	Degrees		Mean squares		
	of	freedom	Body wt. gain	Tibia ash	Toe ash
Calcium					
Linear	C _L	1	2.88*	5.45*	0.35
Quadratic	C _Q	1	0.52	2.68	0.41
Phosphorus					
Linear	P _L	1	28.79**	177.02**	17.28**
Quadratic	P _Q	1	0.24	0.03	0.09
Interaction					
C _L x P _L		1	0.34	7.01*	1.40
Residual		3	0.14	1.94	0.57
Error		9	0.33	0.84	0.30

* and **: Significant at 5 and 1% levels, respectively

containing various combination of calcium and phosphorus are presented in Table 3, and analysis of variance of the data are presented in Table 4. As shown in Table 4, main effect of dietary calcium level and interaction between dietary calcium and phosphorus on the toe ash content were not significant statistically, suggesting that toe ash content varied only in proportion to phosphorus level and that dietary calcium level had little effect on the toe ash content. In contrast, interaction between calcium and phosphorus on the tibia ash content was significant. As shown in Table 3, the trend of increasing tibia ash content in proportion to the increase of dietary phosphorus was observed at 3 dietary calcium levels, and the trend was similar on statistical point of view when dietary calcium level was constant at 0.8 or 1.0%, while the trend, when dietary calcium was at 1.2%, was gentle.

Discussion

The composition of low phosphorus basal diet was so decided that the level of dietary phosphorus as low as possible with ingredients readily available in this country. The diet produced ricket in newly hatched chicks as shown in Fig. 1, and clear breed difference was observed in the susceptibility to ricket. Although the ricket, when not so severe, seems to have little effect on the ash content of the bone, it is better to exclude the chicks having ricket in the biological assay of available phosphorus. This can be achieved by using 7-day-old White Leghorn chicks reared on the restricted feeding program³⁷⁾. The preliminary restricted feeding program may not be necessary in successful bioassay for available phosphorus, but the program has several merits as mentioned in the previous paper³⁷⁾.

The ash contents of the tibia and toe of meat-type chicks were lower than those of White Leghorn chicks fed the same diet as shown in Fig. 3. The findings are in agreement with those reported by SPANDORF and LEONG¹⁸⁾ and GARDINER³⁴⁾. However, the responses of both types of chicks to the changes of dietary phosphorus were almost parallel, indicating that both of the chicks can be used in bioassay of available phosphorus.

The ash contents of the tibia and toe of 7-day-old chicks reached almost constant at 8~12 days after feeding experimental diets of various phosphorus levels as shown in Fig 2. The content increased gradually, while keeping the difference in ash content between the chicks fed diets of different phosphorus levels almost constant, and resulting in two parallel lines. The findings reveal that 10 days of experimental period is satisfactory for bioassay of available phosphorus to obtain similar conclusion to those obtained in bioassay of 3 or 4 weeks of period. Though not presented in detail in this paper, ash content of the chicks fed the experimental diet from one-day-old was also almost constant at 8~10 days after the start of feeding. As mentioned by AMMERMAN *et al.*³⁵⁾, 6 days of period may be too short to get stable assessment of available phosphorus.

Toe ash content is useful criteria for biological assay of available phosphorus as of vitamin D³⁵⁾. Besides the simplicity and time- and labour-saving in determination, toe ash is superior to tibia ash, since dose-response curve of toe ash is linear within wider

range of phosphorus level than that of tibia ash is as shown in Fig. 3 and 5. Correlation coefficient between the tibia and toe of 262 White Leghorn chicks was 0.952 and that of 60 meat-type chicks was 0.929, both being highly significant statistically.

The relationship between dose, *i. e.*, dietary phosphorus level, and ash content in the tibia or toe was linear as shown in Figs. 3, 5 and 6. The findings are agreeable with those reported by GILLIS *et al.*⁶⁾, but not with those reported by SUMMERS *et al.*⁹⁾ and NELSON and PEELER¹⁰⁾, who found linear regression between logarithm of dose and ash content and applied parallel line assay. Correlation coefficients between dose and ash content and between log dose and ash content, calculated by the author using the data presented by SUMMERS *et al.*⁹⁾ were all significant, except one as shown in Table 5. The findings indicate that dose-response as well as log dose-response relationships were both assumed to be linear within the range of added phosphorus from 0.028 to 0.111%. It is not necessary to change the dose into logarithm.

Since in parallel line assay described by SUMMERS *et al.*⁹⁾ availability of phosphorus in the test sample was estimated beforehand, and dietary levels of available phosphorus in the standard and test diets were adjusted to be identical. Under these experimental condition, two regression lines for standard and test diets stood close each other as two lines for tribasic and dibasic calcium phosphates shown in Fig. 3. In such a case, almost identical result will be obtained by either slope ratio assay or parallel line assay. For example, phosphorus availabilities of soft phosphite and phosphoric acid in Expts. 1 and 2, respectively, in the report of SUMMERS *et al.*⁹⁾ were calculated to be 47.5 and 122, respectively, by the slope ratio assay on dose-response lines, while those originally reported were 47.5 and 120, respectively, by parallel line assay on log dose-response lines. A sample with phosphorus of low availability can not be assayed by the parallel line, since the lines are not parallel as shown in Fig. 6. Generally speaking, slope ratio assay is superior to parallel line assay.

Recommendation of the standard procedure for estimation of phosphorus availability

The authors recommend following procedure as the standard procedure for estimation of phosphorus availability.

Preparation of experimental diets: Five diets, a blank containing 0.3% of phosphorus from soybean meal, 2 diets containing 0.15 and 0.3% of added phosphorus from standard phosphorus source, and 2 diets containing about 0.09 and 0.18% of added phosphorus from test material, are prepared, of which composition is as presented in

Table 5. Comparison of correlation coefficients from SUMMERS *et al.* (Poultry Sci., 38, 1168, 1959)

	Dose-response	log dose-response
Expt. 1:		
Calcium phosphate	.991**	.981*
Soft phosphate	.997**	.991**
Expt. 2:		
Calcium phosphate	.984*	.932
Phosphoric acid	.965*	.975*
Soft phosphate	.996**	.978*

* and **: Significant at 5 and 1% levels, respectively.

Table 1. Either tribasic or dibasic calcium phosphate can be served as standard phosphorus. It is desirable to keep dietary calcium constant at 1% by adjusting the level of calcium carbonate.

Chicks and rearing: Newly-hatched chicks are preliminarily fed a conventional chick diet under the restricted feeding program of 10 g per head for initial 3 days and thereafter 3.5 g per head daily. After 7 days of pre-feeding, chicks were grouped into groups of a certain number of chicks, usually 5, having almost the same body weight. Two to three or more replicated groups are allotted to each of the experimental diet. Feed and water are given in free access. After 10 days, chicks are sacrificed. Middle toe from left (or each) foot is excised by cutting through the joint between second and third digital bone. The toes of 5 chicks as a group are dried for 8 hours or until their weight becomes constant at 100°C in air oven, and ashed for 4 hours at 600°C in a furnace. Ash content in dried toe is calculated.

Statistical analyses: Error variance among the replicated estimations of 5 chicks each is estimated. When n test materials are tested in one experiment with r replications, degrees of freedom of error variance is $(2n+3)(r-1)$. Then, phosphorus availability of each test material can be estimated by 5-point slope ratio assay procedure.

Alternative procedures: If necessary, any types of $(2k+1)$ -point slope ratio assay can be applied. Numbers of chicks in a group and of replication can be increased to increase accuracy of assay. Levels of added phosphate from test material other than 0.09 and 0.18% can be adopted within the range less than 0.3%. Chicks in replicated group fed the same diet can be reared as one large group with suitably large feeding space.

Summary

Experimental condition for biological assay of phosphorus availability by chicks was studied. Breed difference was observed in incidence of ricket of one-day-old chicks fed low phosphorus diet containing 0.3% of phosphorus from soybean meal. Ricket could be prevented by using 7-day-old White Leghorn male chicks reared on the restricted feeding program. Ten days of experimental period was satisfactory to get similar conclusion to those obtained by bioassay of 3 or 4 weeks of period. Toe ash content was superior to tibia ash content. Linear relationship between dietary added phosphorus and toe ash content was observed within the range of added phosphorus from 0 to 0.3%.

A simplified bioassay procedure of ten days of feeding period is recommended, which is a slope ratio assay of linear regression lines between added phosphorus and toe ash content.

Availability and its 95% fiducial interval of phosphorus in feed grade dibasic calcium phosphate were 99% and 115~84%, respectively. Those of phosphorus in fish meal were 109% and 127~90%, respectively. Those of phosphorus in yellow corn were 0% and 33~-31%, respectively.

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ヒナによるリンの利用率の測定法の改善

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ヒナによるリンの利用率の生物定量法における実験条件を検討した。初生ヒナに、大豆粕からのリン0.3%を含む低リン飼料を与えると、クル病がみられその発生率は鶏種間で差が認められた。クル病は制限給与して育成した7日齢の白レグ種雄ヒナを使用することにより防ぐことができた。実験期間は10日で十分であって、3週もしくは4週間の生物定量法の場合と同じ結論がえられる。脛骨灰分にもとづくより、趾灰分にもとづく測定のほうがすぐれていた。飼料に添加したリンの含量が、0～0.3%以下の条件でリン添加量と趾灰分との間に直線

関係が認められた。

これらの実験の結果からリン添加量と趾灰分含量との直線関係にもとづき傾斜比法により測定する10日間の簡単な生物定量法を提唱した。

飼料用リン酸2カルシウムのリンの利用率とその95%信頼区間は、99%と115～84%であった。魚粉のリンについては、それぞれ109%と127～90%であり、黄色トウモロコシのリンについては、それぞれ0%と33～31%であった。

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