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Determination of Phosphorus Fractions in Animal Protein Ingredients

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Phosphorus (P) is present in different chemical compounds in animal feeds, and the solubility and digestibility of these different compounds are known to differ significantly. Animal protein ingredients generally have a high P content and are major contributors to total P of feeds for fish and other domestic animals. Estimation of different P compounds in these ingredients could help to improve the accuracy of estimates of digestible P contents of feeds. Bone P and organic P contents were quantified in 32 animal protein ingredients, including 10 fish meals, 14 meat and bone meals, and 8 poultry byproducts meals, using a fractionation protocol. The total P contents of the ingredients ranged from 2.1 to 8.3% on a dry matter (DM) basis. Organic P contents varied between 0.3 and 1.3% of DM. Highly significant ($p < 0.001$) linear relationships were observed between total P and ash and between bone P and ash for all ingredients combined: total P (%) = 0.185 × ash (%) ($R^2 = 0.88$), and bone P (%) = 0.188 × ash (%) − 0.852 ($R^2 = 0.94$). These results suggest that bone P can be easily and reliably estimated on the basis of ash content in animal protein ingredients.

KEYWORDS: Phosphorus; feed; digestibility; composition; diet; animal protein ingredients

INTRODUCTION

Managing phosphorus (P) waste outputs is a key factor for environmental sustainability of animal production operations. The development of effective nutritional strategies to manage P waste outputs requires a detailed understanding of P nutrition (supply, digestion, accretion, excretion) of animals.

Phosphorus is a component of several different types of chemical compounds found in ingredients and feeds. These compounds include hydroxyapatite (bone P), *myo*-inositol hexaphosphate (phytate P), P compounds covalently linked to protein, lipid, and sugar (organic P), and various inorganic phosphate supplements. These compounds are present in various amounts in animal feeds depending on feed formulation and the compositional variability of the ingredients used. Differences in the chemical characteristics and solubility of these compounds are likely to result in different digestion dynamics of P within the animal gastrointestinal tract, and this, in turn, can significantly affect P digestibility. It is consequently necessary to quantify the different P forms in ingredients to better understand and/or predict the digestibility of P in feeds.

Animal protein ingredients (fish meal, poultry byproducts meal, and meat and bone meal) generally have high P contents and often contribute a significant proportion of the total P of feeds for fish and, occasionally, other domestic animals. Animal protein ingredients are produced from a wide variety of raw materials and manufacturing techniques and equipment (*1*, *2*). Consequently, P content and the proportion of chemical compounds in these ingredients may be highly variable, even for a given type of ingredient. A survey of the literature indicates that there are between 16 and 42 g kg^{-1} of P in fish meal, from 25 to 56 g kg^{-1} of P in meat and bone meal, and from 17 to 35 g kg^{-1} of P in poultry byproducts meal (*3*−*8*). Very little information on the proportion of P chemical compounds in these ingredients is available in the literature, although it is well-known that in the body of vertebrates, the majority of P (85−88%) exists as bone P, ∼10−15% is organic P, and only a small amount is present as free ions or soluble inorganic P phosphates (P_i) (*9*, *10*).

Estimates of the digestibility of P for animal protein ingredients are highly variable even for similar ingredients. For example, estimates of apparent digestibility of P in fish meal vary between 17 and 81% for rainbow trout (*6*−*8*, *11*, *12*). Differences in the levels of different P chemical forms could explain part of the variability in the estimates of apparent digestibility of P. Information on the contents of various chemical forms of P in animal protein ingredients would enable better prediction of digestibility of P in feed and/or P waste output by animal production operations (*13*). There have been attempts to estimate bioavailability of P in ingredients and feeds based on chemical extractions (*14*−*17*). A fractionation method was also used for estimates of composition of animal manures (*14*, *18*−*20*). However, limited work has been carried out to quantify specific chemical compounds in animal protein ingredients. There is also a need for simple methods of estimating total P and bone P contents of feed ingredients based on routine chemical analyses (e.g., proximate analysis).

The objectives of the study were to (1) quantify bone P and nonbone P in animal ingredients and (2) determine the relationship among bone P, total P, and proximate analysis parameters.

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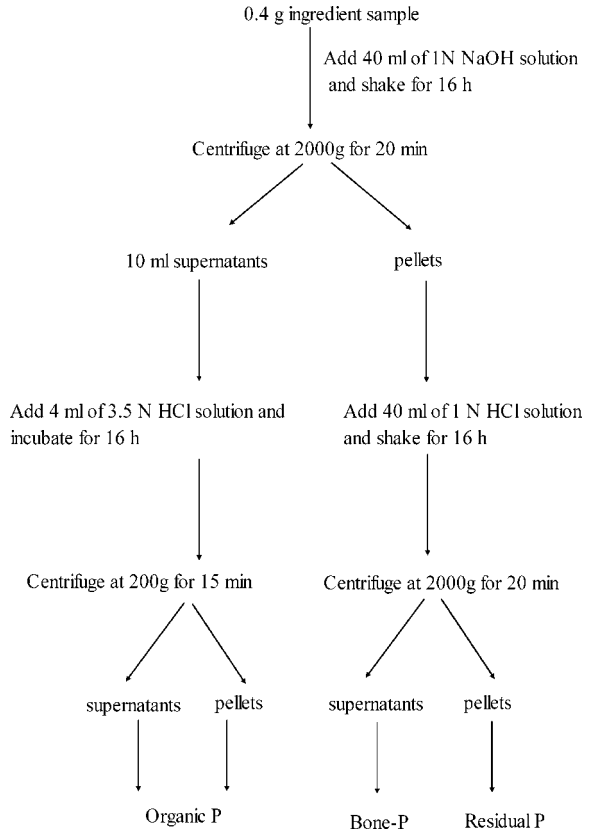

Figure 1. P fractionation protocol.

MATERIALS AND METHOD

Sources of Samples. Thirty-two animal ingredients, including 10 fish meals, 8 poultry byproducts meals, and 14 meat and bone meals, were obtained from various suppliers in North America. These ingredients were selected to cover a wide range of raw materials and finished products for each ingredient type.

Chemical Analyses. Duplicate samples of ingredients were analyzed for proximate composition. Dry matter (DM) was analyzed by heating samples at 105 °C for 24 h. Ash was analyzed according to AOAC gravimetric method 942.05 (*21*). Crude protein (%N × 6.25) was analyzed according to the Kjeldahl method using a Kjeltech 1030 autoanalyzer (Tecator, Höganäs, Sweden). Lipid was analyzed according to AOAC acid hydrolysis method 954.02 (*21*) by a commercial laboratory (AgriFood, Guelph, ON, Canada). A coefficient of variation (CV) of replicates below 5% was considered to be acceptable.

The P fractionation protocol was carried out as detailed in Ruban et al. (*22*, *23*) but with slight modifications (**Figure 1**). Triplicate ingredient samples (0.4 g) were incubated in 1 N NaOH overnight with shaking and then centrifuged. An aliquot of supernatant was incubated in 3.5 N HCl overnight, whereas pellets were incubated in 1 N HCl overnight with shaking, and then centrifuged. The supernatants and pellets were evaporated to dryness on a hot plate. The resulting P fractions included bone P, organic P, and residual P (P resistant to acid and alkaline extraction, and thus unaccounted for in analysis). P contents in animal protein ingredients and fractioned samples were analyzed according to the colorimetric method of Heinonen and Lahti (*24*).

Calculations and Statistical Analyses. The total P content of each ingredient analyzed was compared to the sum of bone P, organic P, and residual P by *t* test. Relationships between all analyzed variables were subjected to linear regression using SAS software (*25*). Probability (p) of <0.05 was considered to be significant.

RESULTS

Table 1 summarizes the results of crude protein, lipid, ash, total P, bone P, organic P, and residual P on a DM basis in fish meals, poultry byproducts meals, and meat and bone meals. Overall, the total P contents of all ingredients samples varied from 2.1 to 8.3%, and ash contents varied from 10 to 37% on a DM basis. The total P contents of fish meals ranged from 2.5 to 4.7% on a DM basis, whereas bone P contents were between 1.4 and 3.5%. Bone P accounted for 53−79% of total P in fish meal. In poultry byproducts meals, total P contents and bone P contents ranged from 2.1 to 3.6% and from 1.2 to 3.1% on a DM basis, respectively. This translated into 60−91% of the total P being present as bone P in poultry byproducts meals. In meat and bone meals, total P content varies from 2.2 to 8.3% of DM, of which between 71 and 93% was bone P. On a DM basis, bone P contents of the 14 meat and bone meals varied between 1.6 and 7.0%. Organic P varied between 0.3 and 1.3% in all ingredients. Residual P represented <2.5% of total P in all ingredients. The difference between total P and the sum of bone P, organic P, and residual P did not exceed 10% in all ingredients and was not significantly different ($p > 0.05$).

Table 1. Contents of Dry Matter (DM), Crude Protein (CP), Lipid, Ash, Total P, and Bone P in Fish Meals (FM), Poultry Byproducts Meals (PBM), and Meat and Bone Meals (MBM)

		% DM						
	DM %	CP	lipid	ash	total P	bone P	org P	resid P
Fish Meals								
FM-1	90.1	78.3	12.4	10.7	2.6	1.4	1.0	0.0
FM-2	85.4	74.0	11.1	12.0	2.5	1.5	1.0	0.0
FM-3	75.6	71.9	10.2	14.5	2.6	1.7	0.9	0.0
FM-4	93.4	66.7	13.6	17.6	3.7	2.7	0.8	0.0
FM-5	92.9	68.3	10.5	19.8	4.7	3.5	1.3	0.1
FM-6	91.1	68.0	9.2	20.8	3.8	2.8	0.8	0.1
FM-7	90.6	68.6	6.1	20.6	3.8	3.0	0.7	0.1
FM-8	92.0	68.1	14.0	17.8	3.4	2.5	0.9	0.0
FM-9	94.2	73.6	10.0	16.2	2.5	1.7	0.7	0.0
FM-10	92.0	73.1	8.8	15.8	2.7	1.8	1.0	0.0
Poultry Byproducts Meals								
PBM-1	96.2	67.6	13.4	14.4	2.7	1.8	0.7	0.0
PBM-2	93.7	68.2	14.7	12.7	2.5	1.8	0.7	0.0
PBM-3	94.1	70.1	16.8	9.8	2.1	1.2	0.8	0.0
PBM-4	98.5	61.4	15.0	18.9	3.4	3.1	0.6	0.1
PBM-5	94.2	68.3	14.9	13.6	2.6	2.0	0.7	0.0
PBM-6	93.6	64.6	10.9	19.7	3.6	3.1	0.5	0.1
PBM-7	96.3	72.0	14.9	13.1	2.6	1.7	0.8	0.0
PBM-8	93.9	69.8	9.4	14.4	2.7	1.9	0.7	0.0
Meat and Bone Meals								
MBM-1	95.0	54.8	13.6	22.3	4.2	3.5	0.6	0.1
MBM-2	96.3	61.8	10.0	22.5	3.5	3.0	0.5	0.1
MBM-3	96.1	54.0	12.8	27.7	4.7	3.9	0.4	0.1
MBM-4	95.1	49.0	11.8	35.5	6.3	5.9	0.3	0.1
MBM-5	96.5	57.0	12.7	23.5	3.5	3.2	0.5	0.1
MBM-6	90.5	57.0	14.3	23.1	4.0	3.3	0.5	0.1
MBM-7	94.5	50.9	12.8	27.8	5.0	4.3	0.5	0.1
MBM-8	95.2	55.2	12.5	24.9	4.0	3.2	0.5	0.1
MBM-9	96.0	45.7	12.1	37.3	8.3	7.0	0.9	0.2
MBM-10	95.6	49.6	11.8	26.9	5.5	4.3	1.1	0.1
MBM-11	95.0	59.8	19.7	13.2	2.2	1.6	0.6	0.0
MBM-12	94.3	50.5	12.0	30.8	5.4	5.0	0.4	0.1
MBM-13	92.2	55.6	10.7	23.8	3.8	3.2	0.5	0.1
MBM-14	95.2	63.7	12.3	21.4	4.0	3.3	0.4	0.1

Figure 2 illustrates the relationship between the analyzed variables. Highly linear relationships ($p < 0.0001$) were observed among bone P (%), total P (%), ash (%), and protein (%) as follows:

$$\text{bone P} = 0.980 \times \text{total P} - 0.711 \ (R^2 = 0.97, p < 0.0001)$$

$$\text{total P} = 0.185 \times \text{ash} \ (R^2 = 0.88, p < 0.0001)$$

$$\text{bone P} = 0.188 \times \text{ash} - 0.852 \ (R^2 = 0.94, p < 0.0001)$$

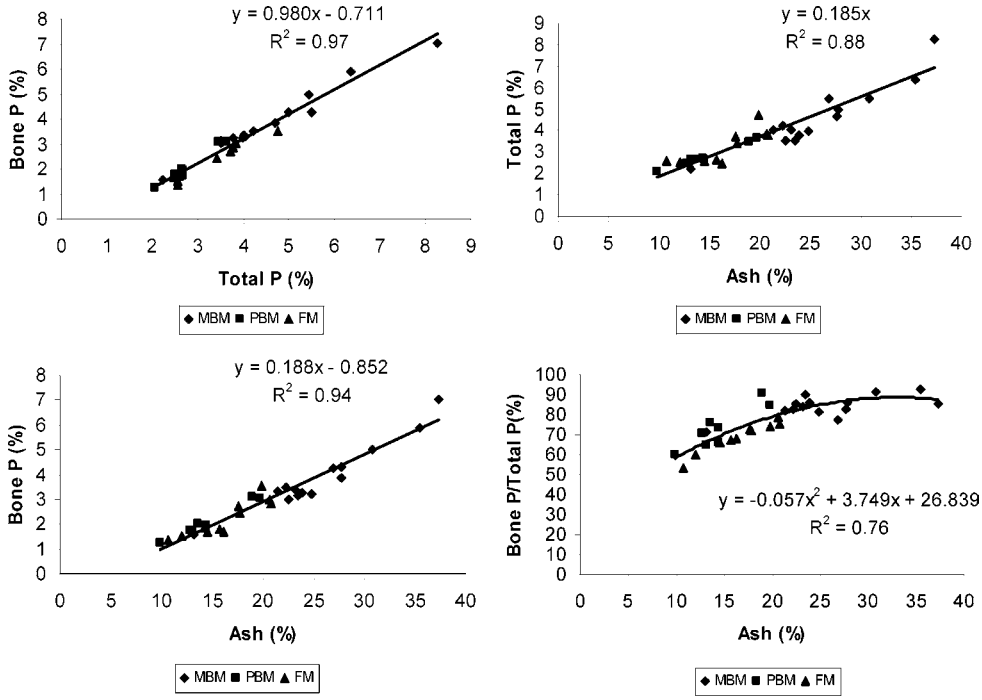

Figure 2. Relationship among bone P, total P, ash, and bone P/total P in meat and bone meal (MBM), poultry byproduct meal (PBM), and fish meal (FM).

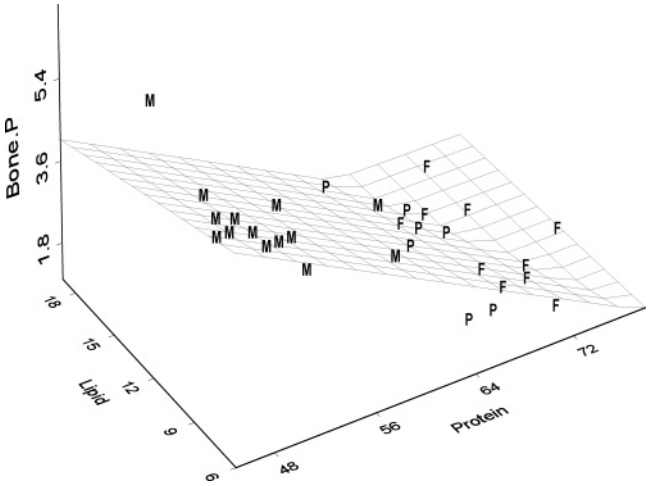

Figure 3. Relationship among bone P (%), protein (%), and lipid (%) in meat and bone meal (M), poultry byproduct meal (P), and fish meal (F). The linear relationship was described as bone P = 13.520 − 0.139 × protein − 0.150 × lipid (R^2 = 0.82).

The relationship between proportion of bone P in total P (%) and ash (%) appeared to be asymptotic and could be in practice described by the following quadratic equation:

$$\text{bone P/total P} = -0.057 \times \text{ash}^2 + 3.749 \times \text{ash} + 26.839 \quad (R^2 = 0.76,\ p < 0.0001)$$

A significant linear equation was obtained to describe the relationship between bone P (%), protein (%), and lipid (%) content as illustrated by **Figure 3** and the following equation:

$$\text{bone P} = 13.520 - 0.139 \times \text{protein} - 0.150 \times \text{lipid} \quad (R^2 = 0.82,\ p < 0.0001)$$

DISCUSSION

In the present study, bone P accounted for 53−93% of total P in the animal protein ingredients analyzed, reflecting the variability of the types and proportion of raw materials used in the manufacturing of these ingredients. Bone is a prominent raw material component in high-ash animal protein ingredients. Bone P content was negatively correlated with protein and lipid contents (**Figure 3**) and positively correlated with ash content (**Figure 2**). The bone P/total P ratio approached an asymptote at high ash levels (**Figure 2**). Organic P content represented a minor proportion of total P content, especially at high ash levels. Residual P represented <2.5% of total P in all ingredients.

The wide variation of bone P content appears to explain the variation of P digestibility of animal byproducts reported in the literature. For salmonid fish, P digestibility ranges from 17 to 81% for fish meal, from 22 to 45% for meat and bone meal, and from 15 to 64% for poultry byproducts meal (*6*−*8*, *11*, *12*, *26*). For swine, P digestibility was in the range of 66−85% for meat and bone meal and 85−90% for fish meal (*27*, *28*). In poultry, P digestibility was reported to be 74% for fish meal and 66% for meat and bone meal for 3-week-old broilers (*29*). Because bone P is generally believed to be less digestible than organic P to fish (*9*) and its digestibility is not additive (*7*), the content of bone P in ingredients and the inclusion level of ingredients in experiment diets will greatly affect P digestibility of an ingredient. The depressing effect of dietary P level on P apparent digestibility in fish (*7*, *30*, *31*) may be primarily due to the limited capacity of the fish gastrointestinal tract to solubilize hydroxyapatite, when diets were formulated with high levels of animal ingredients, rather than through down-regulation of intestinal active transport by high P_i concentration (*32*). Therefore, quantification of different dietary P forms in feeds is needed to better understand and predict apparent digestibility of P.

Analysis of bone P and total P contents of different batches of animal protein ingredients is an expensive and tedious process. The heterogeneous nature of animal protein ingredients, in particular, high-ash meat and bone meal, further complicates analysis. Given the very good relationships between contents of bone P, total P, and ash, our study suggests that bone P content in animal protein ingredients can be easily and reliably

estimated on the basis of total P content or ash content of the ingredients. Our study also suggests that there is no advantage in measuring organic P directly instead of estimating it as the difference between total P and bone P.

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